S [2]Duffield, D. 1987. Investigation of genetic variability in stocks of the bottlenose dolphin (*Tursiops truncatus*) and the loggerhead sea turtle (*Caretta caretta*). Contract Report NA83-GA-C-00036.

Bottlenose dolphin: An important requirement for stock management of the bottlenose dolphin (*Tursiops truncatus*) is the definition and delineation of population boundaries, both in a geographic and a genetic sense. As a considerable number of *Tursiops* are held in display facilities in the United States, a unique opportunity exists to examine the distribution of genetic variability present in animals taken from a variety of geographic locations along the Southeastern United States and from the Pacific. This study compares allelic frequencies and levels of heterozygosity based on electrophoretic analyses of serum and red blood cell proteins for *Tursiops* representative of capture sites extending along the Atlantic coast of Florida, through the Florida Keys, to the Gulf of Mexico and in the Pacific, from Southern California through the Gulf of California. There were no discrete allelic differences between any of the collection sites. Allele frequency and genotypic profile differences provide some evidence for local sub-population differentiation, however there is evidence, as well, for gene flow between collection areas. Adequate biochemical variability exists in *Tursiops* to make this approach a useful tool for examination of the pattern of local area use by bottlenose dolphin herds and for the detection of reproductive exchanges between these groups. It is recommended that *Tursiops* stock management will require describing the sub-population structure of each region, the use of that region overtime by different groups and the evaluation of reproductive exchange between these groups.

Loggerhead sea turtle: This study was designed to test the applicability of biochemical and chromosomal variation analyses for the genetic definition of breeding stocks of the loggerhead turtle, *Caretta caretta*. Blood samples were obtained from loggerhead turtles, either as adults or hatchlings, from three different collection sites along the Southeastern Atlantic Florida coastline. Electrophoretic and chromosomal techniques used in part I. of this study were applied to these samples. No variability was detected for the 15 protein loci examined and although fluorescent R-banding of the chromosomes showed the presence of potentially variable regions in the turtle karyotype, resolution was poor from the blood preparations and variant pattern could not be resolved. These systems did not appear to have productive potential for determining spatial differentiation of stocks in *Caretta caretta*.

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PREFACE

This report was prepared by the author under contract NA83-GA-C-00036 to the National Marine Fisheries Service. Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

INVESTIGATION OF GENETIC VARIABILITY IN STOCKS OF THE BOTTLENOSE DOLPHIN (Tursiops truncatus)

ABSTRACT

An important requirement for stock management of the bottlenose dolphin (*Tursiops truncatus*) is the definition and delineation of population boundaries, both in a geographic and a genetic sense. As a considerable number of *T. truncatus* are held in display facilities in the United States, a unique opportunity exists to examine the distribution of genetic variability present in animals taken from a variety of geographic locations along the Southeastern United States and from the Pacific. This study compares allelic frequencies and levels of heterozygosity based on electrophoretic analyses of serum and red blood cell proteins for *T. truncatus* representative of capture sites extending along the Atlantic coast of Florida, through the Florida Keys, to the Gulf of Mexico and in the Pacific, from Southern California through the Gulf of California. There were no discrete allelic differences between any of the collection sites. Allele frequency and genotypic profile differences provide some evidence for local subpopulation differentiation, however there is evidence, as well, for gene flow between collection areas. Adequate biochemical variability exists in *Tursiops* to make this approach a useful tool for examination of the pattern of local area use by bottlenose dolphin herds and for the detection of reproductive exchanges between these groups. It is recommended that Tursiops stock management will require describing the subpopulation structure of each region, the use of that region overtime by different groups and the evaluation of reproductive exchange between these groups.

This study also examined the genetic variability present in hematologic and chromosomal markers. Hematologies were useful for the detection of offshore *T. truncatus* forms in both the Atlantic and the Pacific and showed evidence for occasional physical as well as reproductive intrusion into coastal populations. Chromosomal variation was extensive throughout the entire sampling. As with the biochemical analysis, common variants were found throughout the sample range. There were differences in variant frequency from area to area, but with evidence of clinal mixing of variants into neighboring areas.

INTRODUCTION

The definition of stock differences in *Tursiops truncatus* remains controversial and of management as well as scientific concern. In addition to purely biological considerations, there are fundamental management needs for estimates of population structure and dynamics and species definition. For example: 1) population parameters, such as size, migratory habits, herd composition and stability, are necessary for decisions regarding the live capture industry in order to avoid stock depletion in areas where animals are collected repeatedly from a limited number of capture sites (Odell et al., 1975); and 2) evaluation of nominal species differences between populations of *T. truncatus* in the Pacific vs. Atlantic, northern vs. southern hemisphere is still not clear. It has been the policy of the International Whaling Commission since 1974 (Mitchell, Subcommittee on Small Cetaceans, IWC, 1975) to treat *Tursiops* as one species composed of sharply defined geographical races. This convention has also been adopted by the Marine Mammal Commission (1976) and there is great need for further examination of these distinctions for proper stock management.

The basis for the controversial nature of population and species definition in *T. truncatus* is evident from examination of data from fisheries records and from tagging, census and

observational studies. In certain Atlantic coastal areas, *T. truncatus* populations are highly migratory. Large schools of up to 600 animals are seen and school composition appears to vary seasonally from approximately equal numbers of males and females to largely disportionate aggregations of either sex (Mead, 1975). Field studies and aerial surveys off the Gulf coast of Florida (Irvine et al., 1977; Wells et al., 1977; Odell, 1977; Wells et al., 1980; Irvine et al., 1981) describe near-shore populations which appear to maintain definable home ranges. Individual herd composition tends to vary in number, from approximately 1-70, and in sex distribution. It would seem that they represent smaller dynamic groups which are actually subunits of a larger population, but the delineation of population boundaries and the level of actual interchange between these subunits has not been determined. The migratory nature of these herds is not known; apparent lack of seasonal migration for *T. truncatus* in Texas and southern Florida has been reported (Gunter, 1942; Moore, 1953 - referenced in Caldwell, 1955), as well as seasonal flux in numbers (Shane, 1980). Additional references to aerial observations of *T. truncatus* in the Gulf of Mexico and in the Pacific are available in Leatherwood (1975) and Odell et al. (1975). In general, these also point to highly mobile herds, ranging in number from 1-175. Subgroups or smaller units of animals which tend to move together as cohesive units can be observed in these larger "herds". Exchange between herds and determination of what comprises the entire home range for these herds in not clear. In the Pacific, comparisons of feeding habits, behavior and cranial measurements suggest that off-shore populations of *T. truncatus* may be distinct from those inhabiting the inshore waters of Southern California, Baja and the Gulf of California (Walker, 1975; Walker, 1981). Very little information is available from any of these populations, Pacific or Atlantic, regarding local herd stability, movement and exchange, although recent efforts are being made in this direction by NMFS studies on Indian River *T. truncatus* populations (Odell and Asper, 1982) and on T. truncatus in the Mississippi Sound area (Solangi and Dukes, 1983).

Observations of *T. truncatus* from regions other than the continental United States are also of interest. *T. truncatus* in the Indian Ocean, for example, appear to be organized into larger social units than *T. truncatus* in the Gulf of Mexico or near-shore Atlantic and Pacific waters. Herd composition is apparently highly variable and the groups tend to stay somewhat further off-shore (Saayman and Tayler, 1973). Based on observations of group size and herd composition and stability in *T. truncatus* off the Argentine coast, Wursig and Wursig (1977) have suggested that herd concepts applicable to most terrestrial mammals may need to be redefined with respect to *T. truncatus* populations. In this study, one particular group of *T. truncatus* was observed over a 21-month period as it passed by a given land observation point. A core unit of five identifiable animals was consistently present. Additional identified animals were with the group for some months, left and were spotted with a new group over 300 km away, subsequently returning to the original group many months later. Although the size of this one herd ranged from 8-22 throughout the study period, 53 different animals were identified with the core unit, joining the core unit individually or in small groups for periods of several days to months before leaving. Both considerable stability and considerable fluidity of subunit structure is evident. It is not clear whether the core unit represents animals with close genetic or kinship ties nor is it clear what comprises the boundaries of the overall population to which this herd belongs. It would certainly seem that it encompasses a considerable area and is characterized by a large degree of individual and group interchange.

On the basis of studies such as these, it is evident that a major problem for future stock management of *T. truncatus* will be that of evaluating to what extent individual populations and those within given coastal vs. offshore regions represent ecologically, geographically and genetically distinct stocks.

Given the current availability of *T. truncatus* in captivity and in breeding programs, a number of these questions of population and stock differentiation in *T. truncatus* lend themselves to comparative genetic analysis. To this end

blood samples were obtained from captive *T. truncatus* representing a variety of collection sites around the east and west coasts of Florida, the Gulf of Mexico and from the Pacific. The object of this study was to make a comprehensive estimate of the amount and distribution of genetic variability within and between sampling areas, based upon electrophoretic, hematologic and chromosomal analyses.

MATERIALS AND METHODS

Animals

Oceanaria in the U.S. and Canada holding bottlenose dolphins were contacted and asked if they would participate in this study by supplying a blood sample from each of their dolphins. All parks with regular bleeding programs agreed most willingly. Samples were received from Sea World, Marineland of the Pacific, Flipper Sea School, MarineWorld, Marinelife Inc., Sea Life Park, the Naval Ocean Systems Center and the Aquarium of Niagara Falls. A total of 140 animals were sampled. The areas in which these animals were collected and the numbers representing each area are given in Table 1. The distribution of capture sites in this study is illustrated in Fig. 1. Sites included 3 areas along the Atlantic coast of Florida, 5 areas along the Florida, Alabama and Mississippi coasts in the Gulf of Mexico and 2 sites in the Pacific, off the Southern California coast and in the Gulf of California (Mexico). The Texas and Florida Panhandle areas were underrepresented because the additional animals from these two areas were involved in active breeding colonies and it was felt that disturbance of the animals for blood sampling could cause too much disruption for the newborn calves. Sample numbers were adequate for comparative genetic analysis from the majority of the other areas. A few stranded animals were also available from sites along the east coast of Florida and the Keys. Blood samples were taken by the parks and shipped air express to Portland for the analysis of protein and chromosomal variation.

Protein Electrophoresis

Red Blood Cell Enzymes: The aim of this study was to compare variable loci for frequency differences between collection areas and to identify any electrophoretic variants specific to one locality and not found in others. Five proteins known from previous studies on bottlenose dolphins (Duffield 1980, 1982) to be polymorphic were selected for intensive analysis. Esterase D (ESD), adenylate kinase (AK), glutamic pyruvate transaminase (GPT) and RBC adenosine deaminase (ADA) were run on starch gels following procedures of Harris and Hopkinson (1976). Isoelectric focusing in polyacrylamide gels was tried to improve resolution of the fifth enzyme, phosphoglucomutase (PGM), following the technique of Sutton (1979). Resolution of the PGM system was significantly improved using this technique and isoelectric focusing was used throughout this study for the evaluation of PGM variants. Isoelectric focusing was also tried on the AK system, but it did not add further clarity and all AK's were run on starch.

An additional battery of RBC enzymes was also examined. These included enzymes previously shown to be monomorphic in *T. truncatus* (Duffield, 1980) and added, to these, the enzymes glyoxalase (GLO) and phosphoglyconate pyruvate (PGP). No variants were found in any of these systems.

Hemoglobin: Hemoglobins were evaluated on cellulose acetate (Helena Laboratories, Beaumont, TX) at a buffer pH of 8.6. They were also evaluated by isoelectric focusing (pH range, 4-8) for the presence of unique variants. The cellulose acetate separations were scored by densitometer to determine relative proportions of the different hemoglobins present in a given animal.

Plasma Proteins: Plasma proteins were evaluated by high resolution agarose electrophoresis (Helena Laboratories, Beaumont, TX). Differences in albumins, haptoglobins and transferrins could be resolved by this procedure. Alpha-1-antitrypsin appeared to be variable, but was difficult to score reliably by agarose electrophesis. Therefore, although alpha-1-antitrypsin genotypes are listed in the genetic profiles, the scoring of alpha-1-antitrypsin variants was felt to be

less accurate than for the other proteins and this protein was not used in the analysis of allele frequency differences. Isoelectric focusing was tried on this locus and again, although variability was evident, the genetics of this system and, therefore, the designation of specific genotype, was not certain. An additional plasma protein locus (C3) was not variable in the animals examined.

Hematology

Hematologic values (hemoglobin concentration, Hb; hematocrit, Hct; and red blood cell count, RBC) of all the animals sampled were requested from each park's health records.

Cytogenetic Analysis

Chromosome preparations were made from leucocyte cultures. Routine blood culturing techniques (Hack and Lawce, 1980) were followed. In order to gain a preliminary idea of the range of chromosomal markers in T. truncatus, a fluorescent R-banding technique utilizing chromomycin A-3 and distamycin A (Schweizer, 1980) was applied. Twenty-eight animals were analyzed, spanning all Atlantic and Pacific collection sites. A minimum of five cells were karyotyped per animal.

Statistical Analysis

Allele frequency differences between collection sites were tested by heterogeneity chi-square analysis with the null hypothesis being that all samples came from the same population (Zar, 1984). The expected population allele frequencies at each locus were based either on the assumption that all samples came from one overall population whose allele frequencies were those of the pooled samples, or upon the allele frequencies in one area compared with all others, reiterated over all collection areas. Deviations between expected and observed genotypes (for the evaluation of statistically significant differences in heterozygosity and the distribution of genotypes) were tested by chi-square. The deviation between observed and that expected under the null hypothesis was considered significant at P 0.05 for all tests.

RESULTS

Protein Electrophoresis

Red Blood Cell Enzymes: The five polymorphic (variable) red blood cell enzymes (PGM, GPT, ESD, ADA, AK) were electrophoretically analyzed and animals scored for homozygosity (having only one of the two electrophoretic forms of the enzyme) or heterozygosity (having both forms of the enzyme). There were no discernible differences in the electrophoretic forms of these enzymes between any of the collection areas sampled in the Atlantic, Gulf of Mexico or the Pacific. The genotypes of the animals are indicated for each locus (enzyme) as homozygous for a "slow" variant (here indicated as "1"), homozygous for a "fast" variant (indicated as "2"), or heterozygous with both variants (indicated as "H"). The scoring system is illustrated in Fig. 2. The enzyme/genotype profiles for each animal are given in Table 2, arranged by region and year collected. Captive-born animals were not included in the allele frequency analysis, but were used to verify the inheritance of allelic forms. Included in Table 2 are sex of the individual, hemoglobin type, hematology, albumin and alpha-1-antitrypsin type, haptoglobin/transferrin type and whether chromosome analysis was done. Unique or unusual variants found in any of the protein systems are starred. Hemoglobin and serum protein variants will be discussed in a following section.

Allele frequencies for each of the polymorphic RBC enzymes are given in Table 3, determined for each region sampled and for the collection as a whole. The regional allele frequencies are illustrated on a map of collection sites in Fig. 3. The most common variant of each enzyme is also indicated, region by region, as well as the individual enzyme/genotype profiles of 4 stranded bottlenose dolphins (indicated in the region in which they stranded). Allele and genotypic frequency distributions will be examined further in the discussion.

Hemoglobins: Electrophoretic comparison of bottlenose dolphin hemoglobins distinguishes 2 basic hemoglobin types. Pacific *T. truncatus* exhibit 2 major hemoglobins (indicated as type "2" in Fig. 2) on both cellulose acetate

electrophoresis (pH 8.6) and isoelectric focusing. The electrophoretically slower hemoglobin represents approximately 70% of the total hemoglobin in the animals, the faster, approximately 30%. Most Southeastern United States *T. truncatus* exhibit a single hemoglobin (type "1", Fig. 2) equivalent in migration to the fast hemoglobin of the Pacific *T. truncatus*. Captive-bred Atlantic or Gulf x Pacific T. truncatus crosses exhibit a distinctive hemoglobin electrophoretic pattern ("H", Fig. 2). Two hemoglobins are present, but the relative concentrations of the two hemoglobins are reversed from those of the Pacific parent. All the Pacific *T. truncatus* in this study exhibited the "2" hemoglobin type. Two Atlantic *T. truncatus*, one an animal stranded live in the Florida Keys and taken into Flipper Sea School, and the other a dead stranding on the ocean-side of the Indian River system, had this "2" hemoglobin type. Two additional animals (a female and calf) stranded in the Cape Canaveral area, prior to this study, also exhibited this "2" hemoglobin type. Four wild-caught Atlantic and Gulf of Mexico *T. truncatus* in this study exhibited the hemoglobin pattern seen in the captive-bred crosses. One was from Mississippi Sound, one from Mobile Bay, Alabama, one from Charlotte Harbor and one from the Florida Keys. These animals are indicated by "H" in Table 2. All other Atlantic and Gulf *T. truncatus* had the single fast, type "1" hemoglobin pattern. Based on the 4 wild *T. truncatus* with the cross-bred hemoglobin pattern among a total of 99 Southeastern U.S. *T. truncatus* examined in this study (93 = type "1", 2 = type "2" and 4 = "H"), there does appear to be occasional reproductive exchange between *T. truncatus* of the 2 basic hemoglobin types throughout the Southeastern U.S. area. However, animals were not collected in a way to address local or temporal patterns or rates of interchange in this study.

Hematologies: Hematologies in bottlenose dolphins can be used to distinguish coastal and offshore forms (ecotypes) of *T. truncatus* (Duffield et al., 1983). These findings suggest that bottlenose dolphins can be separated into coastal and offshore ecotypes based upon hemoglobin levels, packed cell volumes and red blood cell counts, the offshore form having higher levels for all three measures. Captive-bred crosses between coastal x offshore type adults produce animals with intermediate hematologic profiles which would indicate a significant genetic basis for these differences.

In the earlier study (Duffield et al., 1983), coastal and offshore ecotypes were found among Pacific *T. truncatus*, but not in the Atlantic or Gulf of Mexico. Hematologic values for each animal in the present study are included in Table 2.

In the Pacific, all animals collected from the Channel Island area off California were of the offshore hematologic ecotype (Hb = 18 g/100 ml; Hct = 52%; RBC = 4.4 10 /mm). The two Pacific animals from the Gulf of California (Table 2 i.d. = Mex) were of the intermediate ecotype (Hb = 16 g/100 ml; Hct = 45%; RBC = 4.0 10 /mm), suggesting that they were offshore-coastal mixes. In the course of the present study, one Atlantic *T. truncatus* was positively identified as being of the offshore hematologic ecotype. This was the live stranded Florida Keys animal (Hb = 19 g/100 ml; Hct = 53%; RBC = 4.2 10 /mm) being held at Flipper Sea School. This animal also exhibited the "2" hemoglobin pattern of the Pacific *T. truncatus*. It is very likely that the other "2" hemoglobin pattern stranded Atlantic animals (referred to above, and one additional animal stranded during the course of this study in the Indian River area) were of the offshore ecotype based on their morphological and color pattern similarities to the live offshore stranded Flipper Sea School animal. But as they were dead when stranded, hematology could not be evaluated. The majority of the Southeastern ~~United States~~ U.S. *T. truncatus* (including the four with the "H" hemoglobin pattern) had hematologies ranging from typically coastal (Hb = 14 g/100 ml; Hct = 45%; RBC = 3.6 10 /mm) to intermediate. As the Southeastern U.S. *T. truncatus* hematologic records reflect changes in technique from park to park and from earlier to more current techniques, it was hard to reliably distinguish technique variance from animal coastal to intermediate hematologic differences and a more detailed analysis of differences between collection sites could not be effectively made here.

If uniformly measured, however, hematologies provide an additional indication of intrusion of off-shore genotypes and have the potential to reveal regional variation among Southeastern U.S. *T. truncatus*.

Plasma Protein High Resolution Agarose Electrophoresis: Plasma protein electrophoresis was useful in the identification of albumin variants and in the distinction of two basic haptoglobin/transferrin *T. truncatus* patterns (Fig. 2). With respect to the albumin locus, the Pacific *T. truncatus* were either homozygous fast (F) or were heterozygous, exhibiting both a fast and slow variant (H). The Channel Islands offshore ecotype animals were all F (Table 2) and the two intermediate ecotype Gulf of California animals were both H. The Atlantic offshore animal from Flipper Sea School was of the F albumin type like the Pacific offshore animals (the other stranded, potentially offshore, animals could not examined because plasma was not available). All of the other Southeastern U.S. *T. truncatus* (with 2 exceptions) were homozygous for the slow (S) albumin variant. The two exceptions, one *T. truncatus* from the Indian River system and one from the Florida Keys, were heterozygous, exhibiting both fast and slow variants.

Two basic haptoglobin/transferrin patterns (indicated A and B in Fig. 2) could be identified by agarose electrophoresis. All of the Pacific *T. truncatus* (with the exception of one of the Gulf of California animals who appeared to be a mix of the two types) exhibited haptoglobin/transferrin type B. Among the Southeastern U.S. animals, the stranded offshore animal at Flipper Sea School exhibited type B like the Pacific animals, all of the rest (with one exception) were type A. This one, an Indian River animal, exhibited a mix of the two types. It is not clear at this point whether the "mix" haptoglobin/transferrin patterns reflect intrusions of an off-shore pattern or whether they are an additional, rare variant of haptoglobin or transferrin.

Chromosomal Variants

The bottlenose dolphin karyotype has 44 chromosomes (22 pairs). These have been previously described (Duffield et al., 1967; Duffield, 1977; Duffield et al., 1978; Duffield, 1982). We present here the application of a fluorescent R-banding technique (Schweitzer, 1980; Donlon and Magenis, 1983) to characterize the dolphin karyotype. This technique differentially stains a substantial number of variable (heteromorphic) regions in the dolphin karyotype while simultaneously banding the chromosomes for identification of homologues. The R-banded karyotype of a Pacific bottlenose dolphin male is presented in Fig. 4. The chromosomes with major variable regions are indicated.

Chromosome heteromorphisms are variable regions in the chromosomes which vary from individual to individual. They most often correspond to regions of heterochromatin which can show extensive variation within a species and are visualized by a variety of stains. They are considered to be normal variants, have no apparent phenotypic effects, are consistent and stable from tissue to tissue, and are directly inherited. They have been used as genetic markers to characterize individual karyotypes and have been applied to the analysis of the inheritance of chromosome abnormalities and in the determination of paternities in humans (Olson et al., 1983). Cetaceans exhibit considerable amounts of heterochromatin with apparent heteromorphisms between individuals in these regions (Arnason, 1974; Duffield, 1977; Arnason et al., 1980; Duffield, 1985). The inheritance of these heteromorphisms or markers has been established in *T. truncatus* in breeding groups at Sea World, San Diego and Florida (Duffield, 1982).

In cetaceans, the visualization of chromosome marker regions is tremendously enhanced using the R-band fluorescent banding technique. The variable regions scored in this *T. truncatus* study are indicated in Fig. 5. There are 11 chromosome pairs with distinctive variable, heteromorphic regions; 10 autosomes and the male Y chromosome. In four of the autosomes, there are 2 different variable regions, one in the upper (p) arm of the chromosome and one in the lower (q) arm. Each region scored is indicated by an arrow. The range of variation and the scoring system for each chromosome pair is illustrated in Fig. 6.

There were no differences in basic karyotype among any of the 28 *T. truncatus* examined

from the Pacific and the Southeastern United States. There was, however, considerable variation in the heteromorphic regions. So much so, that each animal, based on the heteromorphic patterns shown throughout its 11 variable chromosome pairs, could be given its own individual chromosome signature or identity.

The frequency of the variants for each heteromorphic region on the chromosome are tabulated across collection sites (Table 4). Common variants were found throughout the entire sample range, Atlantic to Pacific. Although the number of animals karyotyped per region in this study precludes a detailed analysis of chromosome marker frequencies, the frequencies of specific variants did vary from collection site to collection site. Their distributions took different patterns: for example, for variants of chromosome marker "3p", the only sites at which one of the variants was found were the Florida Panhandle and Mississippi Sound. For marker "2q" the frequency of the three variants was the same for the Indian River and the Mississippi Sound areas, and showed a clinal change in frequency from the Florida Keys-Miami Beach area up to the Florida Panhandle.

A comparison of the number of markers which were homozygous vs. heterozygous in each collection area is made in Table 5. It is apparent that some chromosome markers are more variable than others. For example, individuals were homozygous for chromosome 3 variants at six out of the eight collection sites while all but five animals of the total sample examined were heterozygous for markers on chromosomes 20 and 21. The pattern of homozygosity and heterozygosity varies from collection area to collection area as did the frequency of the variants. But again, with the numbers of animals karyotyped in this study, these patterns are indicative only of potential trends. Site to site variation in the sum of homozygotes and heterozygotes over all the variable markers is evident. Homozygosity for chromosome markers is higher than heterozygosity in the Indian River, Charlotte Harbor,Tarpon Springs and Florida Panhandle areas, whereas heterozygosity is higher in the Florida Keys-Miami Beach area and in the Pacific sample. Marker homozygosity frequency equals heterozygosity in the sample from the Mississippi Sound. Regional differences in levels of chromosome marker heterozygosity may reflect differences in unit population structure and inter-regional reproductive mixing. However, more sampling is needed within each collection area to address this possibility.

DISCUSSION

The general purpose of this study was to examine the genetic variability of *T. truncatus* from U.S. jurisdictional waters by means of an analysis of blood protein, chromosome and hematologic variants and using captive animals from different collection sites to estimate the degree of genetic uniformity or discontinuity exhibited by *T. truncatus* throughout a wide portion of their U.S. range.

Specific goals were:

1) To assess possible stock differentiation by the presence or absence of genetic similarity between disjunct populations and to estimate the degree of reproductive isolation between herds of adjunct, overlapping and disjunct sampling areas;

2) To set the basis for future genetic comparisons within and between populations of *T. truncatus* not currently available for extensive sampling;

3) To serve as a baseline for comparison of the level of genetic variability with other species which may or may not be similar to *T. truncatus* in population structure and dynamics; and

4) To develop essential comparative data for future use in the evaluation of specific local *T. truncatus* populations.

Assessment of Stock Differentiation

RBC Enzyme, Allele Frequency Analysis

There were no distinctions in the enzyme variants detected for any of the protein systems, between any collection sites sampled. In other words, the various electrophoretic forms of the enzymes were the same in Atlantic, Gulf of Mexico and Pacific populations. There were,

however, differences in the frequencies of the allelic variants between collection sites.

Regional allele frequencies for the RBC loci are compared by capture site in Fig. 7. The capture sites are listed in sequence from Mississippi (MS), along the west coast of Florida (Ala, FPH, TS, CH), through the Keys to the Indian River area, with the Pacific (CI) sample frequencies indicated for comparison. Heterogeneity chi-square analysis based on the null hypothesis that all samples came from the same population (Zar, 1984) detected statistically significant differences in allele frequencies among collection sites for the AK (p 0.001), PGM (p 0.001) and ESD (0.025 p 0.01) loci, suggesting that with respect to these three enzyme systems, the samplings were heterogeneous and did not come from the same population.

Heterogeneity chi-square analyses were reiterated on various combinations of pooled collection site frequencies to gain some idea of where the sample heterogeneity lay. For all three enzymes, the heterogeneity chi-square analyses were no longer significant when the Alabama and Mississippi Sound samples were pooled separately. For ESD, the Charlotte Harbor sample pooled separately as well, and for AK, the Florida Panhandle and Pacific samples pooled independently of Alabama:Mississippi Sound and the rest of the collection sites. Histograms comparing the frequency of allele "1" at each of the three heterogeneous loci are presented in Fig. 8 to illustrate this point.

The patterns of allele distribution at each site for all the enzymes are shown in Fig. 9. Visual comparison of these patterns shows a similar pattern of allele frequency distribution for AK, PGM and ESD in the Alabama and Mississippi Sound samples (although these two sites differ at the ADA locus), a similarity in allele frequency distribution pattern between the Florida Panhandle and the Pacific samples, and some general similarity in pattern between the Indian River-Keys, Miami-Charlotte Harbor-Tarpon Springs sample areas, although differences between the measured frequencies do exist between these latter sites.

The regional samples of *T. truncatus* used here to evaluate similarities and differences in alleles and allele frequencies represent animals taken from the collection areas over several years and, with the exception of the Indian River and the Mississippi and Alabama collection areas, several sites are underrepresented. The data presented were intended to be a preliminary assessment of the genetic variation present and its potential for detection of population or subpopulation differences. As such, the data are not appropriate for more stringent tests of genetic divergence or relative mixing rates. However, given the observed differences in allele frequencies at three of the protein loci and an appearance of possible patterns to the variation in these frequencies, it is recommended that additional sampling of each of the collection regions, done in a more clearly defined time-frame and with more equal sample sizes, will yield population delineation data useful for *T. truncatus* stock management in the Southeastern United States.

Genotype Distributions

The distributions of observed homozygotes and heterozygotes compared with those expected based on the allele frequencies measured at each site were tested at all collection sites and across the entire sampling as a whole by chi-square analysis. Most of the observed genotypic distributions fit Hardy-Weinberg expectations for random-mating with the following exceptions. For the entire pooled data, highly significant decreases in the number of heterozygotes observed vs. expected were detected at the AK locus (p 0.001; 28/104 observed, 46/104 expected) and ADA locus (0.025 p 0.01; 36/105 observed, 52/105 expected). For the individual site samples, a significant decrease in heterozygotes from expected was detected in the Indian River sample for the ADA locus (p 0.001; 4/25 observed vs. 12/25 expected), in the Mississippi Sound for the ADA locus (p .05; observed 6/31, expected 12/31), in the Florida Keys-Miami Beach sample for the ESD locus (p 0.05; 0/7 observed, 3/7 expected), and, in the Florida Panhandle sample for the ESD locus (p 0.05; 1/7 observed, 4/7 expected).

A deficiency of observed heterozygotes compared to the number expected, given random mating expectations based on the allele frequencies measured in a sample of individuals,

can be interpreted as evidence for potential local subdivision of populations (the "Wahlund" effect) as it implies that you have actually sampled across two groups with different allele frequencies and different genotypic distributions of homozygotes for these alleles. This effect is seen here at two loci when the collection site samples are pooled to represent an overall population. This supports the earlier suggestion from the analysis of heterogeneity in allele frequencies that population/subpopulation differentiation exists throughout the *T. truncatus* range sampled in this study. Looking at expectations and "Wahlund" effect within individual collection sites, local subdivision of populations would also be suggested within the Indian River, Mississippi Sound, Florida Keys-Miami and Florida Panhandle areas.

To visually evaluate the partitioning of genotypes within and between the sampled areas, a comparison of individual genotypes per area was made (Figs. 10 and 11). Distributions of the genotypes, especially for the ESD, ADA and AK loci, do not seem to be random in a number of the collection site areas (Fig. 10). There appears to be a division, seen especially in the Indian River and Florida Keys-Miami areas, but present to some extent in the other areas as well, into a "1 1 1" homozygous genotype for these three loci vs. a "2 2 2" homozygous genotype, with various degrees of "mixing" of these at the different collection sites. This is analzyed in Fig. 11 as a comparison of overall genotype scores per site. In this scoring system, a homozygous "1" has a score of 1 for each of these three loci so that an individual who is homozygous "1 1 1" at all three has a total genotype score of 3. A heterozygote scores 2 and a homozygous "2" scores 3. A "H H H" genotype for all three loci would thus have a total genotype score of 6 and a "2 2 2" individual would score 9. The other two loci are certainly variable in each area, but there is no particular pattern to their variation, as seen with regard to the ESD, ADA and AK loci, and they were not used in this particular analysis.

Indian River: Both "1 1 1" and "2 2 2" genotypes have been collected in the Indian River area and the frequency of these totally homozygous individuals exceeds that expected based upon predictions of these combined genotypes made from the allele frequencies (Fig. 11). Coupled with the observation of a Wahlund effect in the Indian River area, it could be argued that two groups of *T. truncatus* with different frequencies of "1" vs "2" alleles use this area. The pattern of use cannot be established in this type of study, but based on the presence of a number of individuals in this sampling who show increased heterozygosity for the three loci together (individuals whose genotype scores are in the 6 and 7 range; 3 out of 19 or approximately 16% of the individuals sampled), reproductive mixing must take place between individuals of the opposing genotypes. In other words, there may be a pattern of local subpopulation differentiation, but there is reproductive exchange between these groups. Stranded animals in this area are distributed in the same genotype combinations.

Florida Keys-Miami: There is a stronger distinction between genotypes in this sampling area between the "1 1 1" and "2 2 2" genotypes. A Wahlund effect was apparent in this sample area as well.

Charlotte Harbor and Tarpon Springs areas: Both genotype combinations are present in these areas, but there are many more animals in the mixed, heterozygous categories (7 of 16, approximately 44%, of the animals sampled), and much less evidence of distinct differences in genotypic types.

Florida Panhandle: This area showed more of a balance of mixed genotypes, although on an individual protein locus basis evidence of a slight Wahlund effect was noted in the allele frequency analysis. More samples from this area would be particularly desirable.

Alabama and Mississippi Sound: These areas exhibited more of the "1 1 1" genotype combination, especially Mississippi Sound, but there was again ample evidence of intrusion of "2" alleles, with approximately 44% of the animals showing mixed, heterozygous genotypes. The Mississippi Sound sample showed a Wahlund effect at the ADA locus, suggesting that local subpopulations may exist in this area; the genotypic analysis suggesting that reproductive mixing is occuring between them.

Pacific: The predominant allele combinations in this small sample taken over a wide geographic range were the "2" alleles, but mixing of genotypes was also evident.

A pattern of distribution of genotypes from the Indian River area through Miami Beach-Florida Keys, along the west coast of Florida (Charlotte Harbor and Tarpon Springs) to the Florida Panhandle, and then in Alabama and Mississippi can be visualized in Figure 11; with the two basic "1 1 1" and "2 2 2" genotypes present from the Indian River through Tarpon Springs (accompanied by some mixed genotypic combinations), the Florida Panhandle showing only mixed genotype combinations, the Mississippi Sound area exhibiting a majority of the "1 1 1" genotype but with mixed genotypic combinations as well and the Alabama area sample showing a mixed genotypic combinations with a predominance of "1" alleles. The picture at this point seems to be one of local subpopulation differentiation, combined with mixing of the different genotypes, and with a clinal change in the frequency with which the "2 2 2" genotype is found moving from the east coast of Florida around to the Mississippi Sound.

Conclusions from Allele and Genotype Frequency Analyses

Based on this sampling of *T. truncatus* taken from several collection areas in the Southeastern United States over a 10 year period, there was genetic evidence of population differences in the distribution of allele frequencies, with some similarity between adjacent collection sites (for example Alabama and Mississippi vs. Charlotte Harbor, Tarpon Springs around the Keys and up to the Indian River System). None of these collection sites had identical allele frequencies at all loci. The Florida Panhandle sample was similar in allele distribution to the Pacific *T. truncatus* sample.

Observed heterozygotes are significantly decreased for the sample as a whole at two loci, further supporting the idea of differentiation of populations thoughout this range. A decrease in heterozygotes was also observed within the Indian River, Florida Keys-Miami, Florida Panhandle and Mississippi Sound samples suggesting local subdivision in these regions.

Comparison of the distribution of genotypes, considering all loci together, suggests that a distinction can be made in several of the collection areas between two basic genotypes. There appears to be a clinal pattern of distribution of the genotypes from the east coast of Florida through the west coast to the Mississippi Sound. In each of the collection areas there is evidence of reproductive exchange between these genotypes based on the intrusion of the alleles into heterozygous genotypes. The actual rate of interchange cannot be determined from this study given the nature of the sampling from each collection area over time and low sample numbers in a number of the locations.

Examining Fig. 11 for the collection sites where the "2 2 2" genotypic combination occurs suggests that the population source/s for the "2" alleles may be along the east coast of Florida and the Keys, intruding into the Charlotte Harbor Tarpon Springs area and the Florida Panhandle, but not so directly into the Alabama or Mississippi Sound areas, where like the Indian River area, there is an increase of the "1 1 1" genotypic animals. The sample from the Pacific shows a significant mixing pattern as does the Florida Panhandle sampling. It is tempting to speculate that either the "1 1 1" genotype pattern or the more heterozygous pattern may be typical of local populations, while the "2 2 2" pattern may be more typical of groups moving through local areas. Further evaluation of the distribution of genotypes in specific areas over time will be of interest in this regard.

Onshore-Offshore Ecotype Differentiation

A difference in genetic profile between onshore and offshore ecotypes can be distinguished and it is similar in both Atlantic and Pacific *T. truncatus*. This profile distinction can be seen in hematologies, hemoglobin electrophoresis, serum albumin and in serum haptoglobin/transferrin type. Offshore animals, both Atlantic and Pacific (defined as offshore by increased hematologies), had the Pacific 2 hemoglobin electrophoretic pattern (70% slow, 30% fast), were homozygous for the fast albumin allele and were of haptoglobin/transferrin type B. In the Atlantic, these offshores were

stranded animals; in the Pacific, both offshore and onshore animals had been collected.

Given these genetic distinctions, mixing of the offshore genotypes into onshore populations can be detected as animals with a hybrid hemoglobin electrophoretic pattern, intermediate hematology, mixed albumin type or mixed haptoglobin/transferrin type. Four Atlantic animals in this sampling showed a hybrid hemoglobin pattern - one from the Keys, one from the Charlotte Harbor area, one from Alabama and one from the Mississippi Sound. Heterozygous albumins were detopected in two additional animals - one from the Indian River area and one from the Keys. One more Indian River animal had a mixed haptoglobin/transferrin type. None of these seven animals were heterozygous for all of these traits at the same time. Therefore they were not the immediate results of crosses between an offshore and an onshore parent, but represented segregation of these variants from previous crosses. In the Southeastern U.S., seven out of 100+ animals sampled probably does not represent a significant mixing rate, but it does indicate that onshore and offshore *T. truncatus* populations are not completely reproductively isolated.

Chromosome Variants

There is a high degree of chromosome marker variability in the *T. truncatus* sampled in this study. For specific questions, such as potential paternity and relationship analysis between specific individuals, this amount of variability makes chromosome analysis a powerful analytical tool. Chromosome marker analyses on a region by region basis, given the number of variable markers in the *T. truncatus* karyotype, is recommended for distinction of related population units. The concentration of homozygosity for certain markers in some areas helps to support the possibility that there may be local differentiation of subgroups in some of these coastal areas, but as with the enzyme frequency analysis, there is also evidence for exchange of variants between areas.

CONCLUSIONS

1) Based on the distribution of allele and genotype frequencies, this study presents evidence for local population or subpopulation differentiation among Southeastern U.S. *T. truncatus*, but with indications that these populations or subpopulations are not reproductively isolated from one another.

The decrease of observed vs. expected heterozygotes over the total sample area and in some of the collection regions supports the possibility of local differentiation among these *T. truncatus*. The absence of absolute allele variant differences between collection sites and the patterns of genotype distributions within and between sites are both suggestive of gene flow.

2) This sampling of *T. truncatus*, based on over ten years of collections from a variety of sites distributed throughout the Southeastern management area, suggests that the patterns of distribution of alleles and genotypes vary with collection site. The present study, however, cannot be used to estimate the exact degree of area differentiation or mixing rates as the numbers of individuals which had been taken from neighboring collection sites were not always sufficient for numerical tests of population differentiation or distance measures, nor were the animals always sampled from these regions in a given time-frame. It is recommended that using allele and genotype frequency analysis for estimation of regional population or subpopulation differentiation, area use and reproductive mixing rates can effectively be made for stock management decisions concerning Southeastern U.S. *T. truncatus* by utilizing a series of discrete samplings throughout each region over a period of time. This would allow appropriate statistical testing of within vs. between sample variance, both in and between management areas.

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INVESTIGATION OF GENETIC VARIABILITY IN THE LOGGERHEAD SEA TURTLE (Caretta caretta)

ABSTRACT

This study was designed to test the applicability of biochemical and chromosomal variation analyses for the genetic definition of breeding stocks of the loggerhead turtle (*Caretta caretta*). Blood samples were obtained from loggerhead turtles, either as adults or hatchlings, from threedifferent collection sites along the Southeastern Atlantic Florida coastline. Electrophoretic and chromosomal techniques used in part I. ofthis study were applied to these samples. No variability was detected for the 15protein loci examined and although fluorescent R-banding of the chromosomesshowed the presence of potentially variable regions in the turtle karyotype, resolution was poor from the blood preparations and variant pattern could not beresolved. These systems did not appear to have productive potential fordetermining spatial differentiation of stocks in Caretta caretta.

INTRODUCTION

Caretta caretta, the loggerhead sea turtle, is a highly migratory marine species. Parts of this species' biology, ecology and reproductive migration have been determined, but much is still unresolved. Research on the loggerhead sea turtle (*Caretta caretta*) has been directed towards the nesting activity of female loggerheads (Caldwell, 1959; Carr and Ogren, 1959; Kaufmann, 1975; Talbert et al., 1980), hatchling behavior and movements (Bustard, 1979; Pritchard, 1979; Stoneburner et al., 1982) and to a variety of developmental and physiological parameters (e.g.: O'Hara, 1980; Kraemer and Bennett, 1981; Grassman and Owens, 1982). Population data on the loggerhead, as well as on the green and related sea turtles, have been collected through mark-recapture studies of both adults and immatures, beach surveys of female nesting areas, and aerial census methods (Carr, 1967; Koch etal., 1969; Carr and Carr, 1972; Mendonca and Ehrhart, 1982; Musick et al., 1983; see also symposia publications - Behavioral and Reproductive Biology of Sea Turtles, 1980; Biology and Conservation of Sea Turtles, 1981). Satellite telemetry of loggerhead sea turtle movements has been reported (Stoneburner, 1982). Movements, concentrations and habitat use have been analyzed from stranding and incidental mortality records (Lutcavage and Musick,1985). Population and demographic parameter models have been suggested (see ~~for ex.~~ Richardson and Richardson, 1981; Frazer, 1984). However, a continuing directive to monitor nesting populations of loggerhead turtles in the southeast U.S., has created a need both for more comprehensive population data and for improvement of survey, population assessment and tracking techniques.

One requirement for the management of sea turtles is the further definition of breeding stocks or populations. These are currently arbitrarily defined by location of nesting beaches and the biological basis of these geographic and political boundaries needs to be examined. There has, as yet, been no application of genetics techniques (electrophoretic, chromosomal or DNA) to the evaluation of population or stock differentiation in loggerheads. As it has been hypothesized that mature animals return to their natal beach sites for breeding and nesting, the genetic distinction of disjunct nesting stocks might indeed be possible if informative genetic systems can be identified.

Electrophoretic and chromosomal techniques developed in part I. of this study to evaluate stock differentiation in the bottlenose dolphin

(*Tursiops truncatus*) are applied to a selected sampling of loggerhead turtles from three southeast Atlantic coastal areas to assess the potential usefulness of these two genetic analyses for future loggerhead stock differentiation studies.

MATERIALS AND METHODS

Animals

Loggerhead sea turtle blood samples were obtained from field tagging studies in the Central Florida, Indian River system and from the Cape Canaveral area, as well as from hatchlings being maintained in the Florida Keys. The following samples were received for analysis:

Source/ Location	Date(1984)	No. of Samples	Anticoagulant
P. Raymond/ Central Fl, Indian R.	5/24-6/22	26	EDTA
P. Raymond/ Central Fl, Indian R	6/26-7/18	14	EDTA & Heparin.
P. Raymond/ Central Fl, Indian R.	8/08-8/13	6	Heparin
P. Raymond/ Central Fl, Indian R.	9/07	2	Heparin
N. Thompson/ Cape Canaveral	12/14	9	Heparin
D. Odell/ Florida Keys	5/29/85	23	Heparin
Total animals sampled:		80	

Sampling

Blood samples were drawn by heart puncture (Stephens and Creekmore, 1983) into EDTA or heparinized vacutainer tubes. They were refrigerated and shipped on ice to Portland State, usually within one week of sampling.

Electrophoresis

A number of red blood cell and plasma proteins were scanned for the presence of allozyme variants. The red blood cell proteins examined were hemoglobin (Hb), red cell acid phosphatase (AP), phosphoglucomutase (PGM), RBC adenosine deaminase (ADA), RBC esterase D (EsD), adenylate kinase (AK), and glutamic pyruvate transaminase (GPT). These systems were run on starch gel following techniques described in Harris and Hopkinson (1976). Phosphoglucomutase was also run on an isoelectric focusing gel (Sutton, 1979). Plasma protein systems analyzed were general protein (GP), group-specific complement (Gc) and transferrin (Tfr). These were run using an isoelectric focusing technique adapted from Hoste (1979).

Cytogenetic analysis

Chromosome cultures were tried on 15 unhemolyzed heparin blood samples. They were grown at room temperature, 28 C and at 37 C in commercial RPMI media, supplemented with 25% fetal calf serum, phytohemagglutinin (PHA) and antibiotics. Chromosome preparations were R-banded following the technique of Schweizer (1980).

RESULTS

Blood Sampling

We experienced a problem with the blood samples drawn into EDTA anticoagulant. These samples were hemolyzed and did not survive shipment. All subsequent samples were taken in heparin. Although occasion hemolysis was experienced, most samples taken in this anticoagulant were stored and shipped successfully.

Electrophoresis

No activity was found for the red blood cell systems ACP and ADA in any of the turtles examined. Two additional red blood systems, PGM and GPT, were detected as an unresolved smear. Adjusting buffer systems and pH ranges did not improve resolution of these enzymes, but there were no apparent migration or band size differences between individuals. Esterase D exhibited a single band on the gel, with no differences between individuals. Adenylate kinase was resolved into a three band system, with no differences between in-

dividuals. A large number of plasma protein systems were visualized on the isoelectric focusing gels. No differences were seen between any of the samples for any of the general protein bands or for Gc; and in the transferrin system all samples were identical, with the single exception of one untagged female from the Indian River area.

Cytogenetic Analysis

The best white blood cell growth was seen at 28 C. At best, however, there was very little cell growth obtained by white blood cell stimulation. Fluorescent R-banding showed the presence of several bright, potentially variable, regions in the chromosomes of the loggerhead, but these could not be resolved given the overall poor quality of the chromosome preparations.

DISCUSSION

Electrophoresis

Approximately 15 blood protein loci were evaluated in this study of loggerhead turtles sampled from three sites along the southeast Atlantic coastline. Essentially no variability was detected in any of the protein systems examined, except for one possible electrophoretic migration difference in a plasma transferrin in one untagged and unidentified animal. For this set of biochemical characters, all of the turtles exhibited the same phenotype. These particular systems would therefore appear to be of little use in the estimation of genetic differences between nesting sites.

In a study of turtle phylogeny comparing 22 species of batagurine turtles and 2 species of emydine turtles (the family Emydidae, as compared to the sea turtles, family Cheloniidae), 11 enzymatic proteins were compared (Sites et al., 1984). They found that while the electrophoretic form of the protein differed between species, many species were homozygous at all loci, as seen in this survey of red blood cell proteins in the loggerhead. However, a few of the species exhibited variability at a number of the protein loci, suggesting that one cannot automatically presume that biochemical genetic variability will not be a useful tool for the analyses of population differentiation in turtles in general. The enzyme systems showing the best potential for intraspecific variation were; aspartate aminotransferase, phosphoglucomutase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, and glucosephosphate isomerase - enzymes routinely electrophoresed using tissue extracts (liver, heart and kidney). Seven of ten protein loci were found to be variable (polymorphic) in a study of local population differentiation in the yellow-bellied slider turtle (Scribner et al., 1984). Glutamate oxalate transaminase, phosphoglucomutase, 6-phosphogluconate dehydrogenase and glucophosphate isomerase were the most highly variable (again in tissue extracts). It was proposed that the disjunct nature of suitable habitat could account for this level of local population genetic differentiation, although some gene flow between populations was also felt to occur between neighboring populations.

Although genetic heterogeneity over short geographic distances can be a common occurence in many species and has been detected in some marine mammal species (*Tursiops truncatus*, pers. obs.), it is not a necessary consequence of spatial differentiation. Electrophoretic loci that are variable in the bottlenose dolphin, are not variable in other marine mammal species such as the beluga and the harbor seal (work in progress). Low biochemical genetic variability has also been reported for the walrus, the harp seal and the hooded seal (Simonsen et al., 1982a; Simonsen et al., 1982b), as well as for the elephant seal (Bonnell and Selander, 1974). The reasons for low biochemical genetic variability are not known and may be different for each species. The conclusions to be drawn from the evaluation of the red blood cell proteins surveyed in this study would be that biochemical genetic variability is low in this species, at least for these enyzme systems in the blood. It may be that variable expression of these and additional systems will be found in other tissues, not available in these live studies, or that additional loci not tested here are variable in blood. However, the lack of variants in the isoelectric

focusing screening of general protein systems in the plasma and the lack of variants in the red blood cell loci examined here, suggests that this tool may not be a productive one for the evaluation of spatial differentiation of stocks in the loggerhead sea turtle (*C. caretta*).

Chromosomes

The cytogenetic analysis of loggerhead sea turtle bloods was considered a preliminary assessment of the feasibility of growing this tissue and an evaluation of whether obvious chromosomal markers were present. The loggerhead has a karyotype with a diploid number of 56. There are apparently no sex chromosome differences and the various species of the Fm. Cheloniidae are indistinguishable by G-banding pattern (Bickham and Carr, 1983).

Chromosome studies in turtles have been traditionally done on fibroblasts cultured from heart muscle (Sites et al., 1979; Bickham et al., 1980; Carr et al., 1981). This study would suggest that bloods could be used for chromosome analysis, but that additional refinement of the technique will be necessary to obtain adequate numbers of preparations for marker analysis. The advantage of using blood culture techniques is merely that an animal need not be sacrificed. Athough no differentiation between individuals and species in the Cheloniidae was seen on G-banding (Bickham and Carr, 1983), the preliminary R-banding done here gave the impression of several possible marker areas, heterochromatic regions showing strong R-band fluorescence. The application of chromosome marker analysis in the sea turtle, however, will depend on the development of adequate culture techniques for live turtles, and may not be particularly cost- effective at this point for use in stock differentiation studies.

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TABLE 1. Collection sites represented by this study. The number of animals and the contributing parks are indicated.

Location	N	Contributors
ATLANTIC		
Florida east coast		
Indian River area (IR)	25	Sea World, Flipper Sea School
Miami Beach area (MB)	2	Marineland of the Pacific, Oceanworld
Florida Keys (FK)	3	Sea World, Flipper Sea School
Florida west coast		
Charlotte Harbor (CH)	10	Sea World
Tampa Bay/Tarpon Springs (TS)	6	Sea World, Oceanworld
Florida Panhandle (FPH)	7	Sea World, Flipper Sea School
Upper Gulf of Mexico		
Alabama/Mobil Bay (Ala)	12	Marinelife Inc.
Mississippi Sound (MS)	31	Marinelife Inc., Sea World, Marineworld, NOSC, Aq. of Niagara Fall
Texas	1	Marineworld
PACIFIC		
So. California/Channel Islands (CI)	4	Sea World
Gulf of California (Mex)	2	Sea World
Hawaii	6	Sea Life Park, NOSC
CAPTIVE-BRED ANIMALS	27	Flipper Sea School, Marineland, Sea World, NOSC
	136	

Four additional stranded bottlenose dolphins (3 Atlantic, east coast of FL and Keys; 1 Pacific, San Diego) were evaluated.

TABLE 2: Individual genetic profiles by location. RBC enzyme genotypes are indicated as "1" = homozygous for the slow variant of that enzyme, "2" = homozygous for the fast variant, and "H" = heterozygous and exhibiting both variants. Hemoglobins are scored as "1" for the single hemoglobin type characteristic of most Atlantic Tursiops, "2" for the two hemoglobin type typical of the Pacific Tursiops, and "H" for the hemoglobin type seen in crosses between parents of the two different types. Albumin and alpha-1-antitrypsin ( ) are scored as "S" = homozygous for a slow variant, "F" = homozygous for a fast variant and "H" = heterozygous and having both variants. The haptoglobin/ transferrin electrophoretic pattern is indicated as either being of an "A" or "B" type or mix of the two (A/B). A "+" indicates that chromosome analysis was done on that animal. An * besides a letter or number, indicates that it is a rare or unusual variant for that system.

INDIAN RIVER

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
IR-1	1972	M	2	H	1	1	H	1	14	44	3.6	S	F	A	+
IR-2	1972	F	2	H	1	1	1	1	15	45	3.4	S	H	A	+
IR-3	1974	M	2	2	1	1	H	1	16	47	3.7	S	F	A	+
IR-4	1977		2	H	H	H	H	1	13	39	3.3	S	F	A	
IR-5	1977		H	2	1	1	1	1	15	42	3.7	S	H	A	
IR-6	1977	F	2	H	1	1	1	1	16	47	4.1	S	F	A	
IR-7	1978	F	2	H	H	H	H	1	14	41	3.6	S	F	A	
IR-8	1980	F	2	H	1	1	1	1	13	38	3.4	S	H	A	
IR-9	1980		2	2	2	2	2	1				S	H	A	
IR-10	1980	F	2	2	1	1	1	1	15	44	3.5	S	H	A	
IR-11	1980		2	H	2	2	2	1				S	H	A	
IR-12	1980		2	2	2	2	2	1	15	44	3.4	S	F	A	
IR-13	1980		H	2	H	2	2	1				S	H	A	
IR-14	1980		2	2	H	2	2	1				S	S	A	
IR-15	1980		H	2	H	2	2	1				S	S	A	
IR-16	1980		2	H	2	H	H					H	H	A/B*	
IR-17	1980		2	2	1	1	1	1				S	H	A	
IR-18	1981	F	H	H	1	1	1	1	14	38	3.4	S	F	A	+
IR-19	1983	F	2	2	1	1	1	1	15	44	3.6	S	F	A	+

TABLE 2

Table 2 (cont.)

INDIAN RIVER (cont.)

IR-20st	1982	H	H	H	1	2	
IR-21st	1982	H	H	1	1		
IR-22st	1980	2	2	H	2	H	
IR-23st	1980	H	2	H	2	H	
IR-24st	1982	2*	H	2	H	2	
IR-25st	1980	2	2	2	2	2	2*

MIAMA BEACH

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
MB-1	1964	M	2	2	1	1	1	1	16	50	3.7	S	H	A	+
MB-2	1964	F	2	2	1	1	1	1	14	44	3.5	S	F	A	
MB-3st			H	1	1	H	H	1				S	H	A*	

FLORIDA KEYS

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
FK-1	1972	M	H	2	2	2	2	1	14	43	3.6	S	S	A	+
FK-2	1973	F	H	H	1	1	1	1	15	44	3.8	H*	H	A	
FK-3	1973	M	H	2	2	2	H	H*	16	43	4.0	S	S	A	+
FK-4st	1982	M	2	2	2	2	H	2*	19	53	4.2	F*	H	B*	+

CHARLOTTE HARBOR

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
CH-1	1975	M	2	2	2	2	2	1	14	42	3.5	S	H	A	
CH-2	1975	M	H	2	1	H	1	1	14	40	3.3	S	H	A	+
CH-3	1975	F	H	2	2	2	2	1	14	39	3.4	S	S	A	+
CH-4	1975	F	2	H	H	H	1	1	14	42	3.5	S	H	A	
CH-5	1975	F	2	2	H	1	1	1	14	42	3.5	S	S	A	+
CH-6	1977	M	2	2	1	1	1	1	15	45	3.9	S	S	A	+
CH-7	1977	M	2	2	H	H	H	H*	15	44	3.9	S	F	A	
CH-8	1977	F	2	2	2	H	H	1	14	41	3.5	S	F	A	
CH-9	1977	M	2	2	2	2	2	1	16	46	3.8	S	H	A	+
CH-10	1977		H	2	2	H	H	1	14	41	3.3	S	F	A*	

Table 2 (cont.)

TARPON SPRINGS/TAMPA BAY

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
TS-1	1975	F	H	2	1	1	H	1	16	47	3.8	S	H	A	+
TS-2	1980	F	H	2	1	H	H	1	14	40	3.3	S	H	A	+
TS-3	1980		H	H	H	H	H	1	14	42	3.5	S	H	A	
TS-4	1980	F	2	2	2	2	2	1	16	45	3.8	S	F	A	
TS-5	1980		2	2	H	2	H	1				S	H	A	
TS-6	1980	F	H	2	1	1	1	1	13	39	3.8	S	H	A	+

FLORIDA PANHANDLE

Table 2 (cont.)

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
FPH-1	1969	F	H	2	H	2	2	1	15	42	3.7	S	S	A	+
FPH-2		M	2	2	1	H	H	1	15	44	3.4	S	H	A	+
FPH-3		F	H	2	2	H	H	1	16	45	3.4	S	H	A	
FPH-4	1970	M	H	2	2	H	H	1	16	45	3.9	S	F/S	A	
FPH-5	1971	F	2	2	1	H	2	1	16	45	3.8	S	F/S	A	+
FPH-6	1971	F	H	2	2	H	2	1	14	39	3.4	S	S	A	+
FPH-7	1977	M	2	2	1	1	H	1	17	50	3.6	S	H	A	+

ALABAMA

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
Ala-1	1984	M	2	2	1	H	1	1	14	40	3.2	S	H	A	
Ala-2	1984	M	2	H	1	H	1	1	13	40	3.6	S	H	A	
Ala-3	1984	M	2	2	H	2	1	1	15	43	3.6	S	H	A	
Ala-4	1984	M	2	2	H	2	1	1	14	40	3.2	S	H	A	
Ala-5	1984	M	2	2	H	2	1	1	14	42	3.3	S	H	A*	
Ala-6	1984	F	2	2	1	H	1	1	14	42	3.5	S	F	A	
Ala-7	1984	F	2	2	1	H	1	1	13	39	3.2	S	H	A	
Ala-8	1984	F	2	2	H	H	1	1	15	45	3.6	S	H	A	
Ala-9	1984	F	2	2	H	2	1	1	15	43	3.4	S	H	A	
Ala-10	1984	F	2	2	1	1	1	1	15	45	3.5	S	H	A	
Ala-11	1984	M	2	H	1	H	1	H*	15	45	4.0	S	F	A	
Ala-12	1984	F	2	2	H	H	1	1	12	35	2.9	S	H	A	

MISSISSIPPI SOUND

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
MS-1	1970	F	2	2	1	1	2	1	15	45	3.8	S	H	A	
MS-2	1972	M	2	2	1	1	1	1	15	42	3.7	S	H	A	
MS-3	1972	M	2	2	1	1	H	1	16	46	3.9	S	F	A	+
MS-4	1972	M	2	H	1	1	1	1	14	42	3.6	S	S	A	+
MS-5	1978	F	2	2	1	1	1	1	15	45	3.4	S	H	A	
MS-6	1980	M	2	1	1	1	1	1	14	42	3.4	S	F	A	
MS-7	1983	M	2	2	1	1	1	1	15	46	3.7	S	H	A	
MS-8	1983	F	2	2	1	1	1	1	15	46	3.5	S	H	A	
MS-9	1983	F	2	2	1	1	1	1	15	46	3.9	S	H	A	
MS-10	1983	M	2	2	H	2	H	1							+
MS-11	1983	F	2	H	1	1	1	1	16	48	4.1	S	H	A	
MS-12	1983	F	2	2	1	1	1	1	15	44	3.9	S	H	A	
MS-13	1984	M	2	H	H	2	1	1	14	43	3.5	S	F	A	
MS-14	1984	M	2	1	1	H	1	1	15	44	3.8	S	F	A	
MS-15	1984	M	2	H	H	2	1	1	14	42	3.5	S	H	A	
MS-16	1984	M	2	2	H	2	1	1	14	41	3.4	S	H	A	
MS-17	1984	M	2		2	H	1	1	14	43	3.7	S	H	A	
MS-18	1984	M	2	1	H	H	1	1	14	44	3.6	S	H	A	
MS-19	1984	M	2	H	H	2	1	1	13	38	3.6	S	H	A	
MS-20	1984	F	2	2	H	2	1	1	14	42	3.8	S	H	A	
MS-21	1984	F	2	2	H	H	1	1	14	42	3.6	S	F	A	
MS-22	1984	F	2	2	H	H	1	1	14	42	3.6	S	H	A	
MS-23	1984	F	2	2	H	H	1	1	14	42	3.4	S	F	A	
MS-24	1984	F	2	2	1	1	1	1	15	44	3.5	S	H	A	

Table 2 (cont.)

MISSISSIPPI SOUND(con't)

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES				Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
MS-25	1984	F		2	1	1	1	1	14	43	3.6	S	H	A	
MS-26	1984	F		2	1	1	1	1	16	45	3.7	S	H	A	
MS-27	1984	M		2	1	1	1	1	14	45	4.3	S	H	A	
MS-28	1984	M		H	1	1	1	1	15	43	3.7	S	H	A	
MS-29	1984	F		2	1	1	1	H*	15	44	3.6	S	H	A	
MS-30	1984	F		H	1	1	1	1	16	46	3.8	S	H	A	
MS-31	1984	F		2	1	1	1	1	15	43	3.7	S	H	A	

Table 2 (cont.)

TEXAS

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES				Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
Tex-1	1978	M	2	2	2	H	1	1	15	45	3.4	S	H	A	

CHANNEL ISLANDS and MEXICO

ANIMAL #	CAPTURE YR.	SEX	RBC ENZYMES					Hb	HEMATOLOGIES			SERUM PROTEINS			CHROMOSOMES
			PGM	GPT	ESD	ADA	AK		Hg (g.)	Hct (%)	RBC (#)	Alb.	∝1	Hp./Tr.	
CI-1	1977	F	H	2	2	2	2	2	20	56	4.8	F	H	B	
CI-2	1978	F	1	2	1	H	H	2	18	47	4.5	F	H	B	
CI-3	1978	M	2	2	1	H	2	2	18	51	4.4	F	H	B	+
CI-4	1978	F	H	2	H	H	H	2	18	52	4.1	F	H	B	
SD-1st	1984	F	H	2	H	H	H	2				F/S	H	B*	
Mex-1	1968	F	2	2	H	H	H	2	16	44	3.9	F/S	H	A/B*	+
Mex-2	1971	M	2	2	H	H	H	2	16	46	4.1	F/S	H	B	+

Table 2 (cont.)

TABLE 3. RBC ENZYME ALLELE FREQUENCIES

	ALLELE FREQUENCIES									
	PGM		GPT		ESD		ADA		AK	
REGION (N)	1	2	1	2	1	2	1	2	1	2
Indian River (25)	.14	.86	.24	.76	.60	.40	.56	.44	.48	.52
Keys:Miami Beach (7)	.29	.71	.21	.79	.57	.43	.50	.50	.64	.36
Charlotte Harbor (10)	.15	.85	.05	.95	.35	.65	.45	.55	.55	.45
Tarpon Springs (6)	.33	.67	.08	.92	.67	.33	.50	.50	.50	.50
Fl Panhandle (7)	.29	.71	0	1	.50	.50	.50	.50	.29	.71
Alabama (12)	0	1	.08	.92	.75	.25	.38	.62	1	0
Mississippi Sd (31)	0	1	.22	.78	.81	.19	.71	.29	.94	.06
Pacific (7)	.35	.65	0	1	.57	.43	.43	.57	.36	.64
OVERALL ALLELE FREQUENCY (105)	.13	.87	.15	.85	.65	.35	.55	.45	.67	.33

The Florida Keys and Miami Beach samples are combined here, as well as all the Pacific samples, in order to have enough individuals in these areas for statistical analysis.

TABLE 4: Frequency of chromosome variants in different collection areas. These are tabulated by variable marker region on each chromosome. For some markers there were only two common variants, for some there were three and for one marker there were four variants found. Variant "1" is given first, "2" second, "3" third and 4" fourth.

Chromo.	COLLECTION SITES							TOTAL
Marker	IR	FK.MB	CH	TS	FPH	MS	PAC	SAMPLE
1p	.33/.67	.67/.33	.62/.38	.75/.25	.30/.70	.75/.25	.67/.33	.55/.45
1q	.2/.8/ 0	0 /.8/.2	.6/.2/.2	0 /.8/.2	0 /.9/.1	.1/.8/.1	.2/.5/.3	.1/.7/.2
2p	.2/.3/.5	.3/.1/.6	.6/.2/.2	.8/.2/ 0	.4/.5/.1	.7/.3/ 0	0 /.5/.5	.4/.3/.3
2q	.2/.6/.2	0 /.6/.4	0 /.5/.5	0 /.8/.2	.1/.6/.3	.3/.4/.3	.2/.6/.2	.1/.5/.4
3p	1/0	1/0	1/0	1/0	.33/.67	.50/.50	1/0	.90/.10
3q	.60/.40	.75/.25	1/0	1/0	.62/.38	1/0	.75/.25	.80/.20
4p	.87/.13	.75/.25	.62/.38	.67/.33	.40/.60	.83/.17	1/0	.70/.30
4q	1/0	.62/.38	.90/.10	1/0	.87/.13	1/0	.75/.25	.86/.14
5p	.83/.17	.67/.33	.7/.1/.2	.83/.17	.69/.31	1/0	0/1	.7/.2/.1
17q	.30/.70	.33/.67	.50/.50	.83/.17	.14/.86	.25/.75	.50/.50	.41/.59
18p	.4/.5/.1	.6/.4/ 0	.3/.6/.1	.5/.5/ 0	.2/.6/.2	.6/.2/.2	1/ 0/ 0	.4/.5/.1
19p	0 /.7/.3	.1/.8/.1	.2/.5/.3	.3/.6/.1	0 / 1/ 0	0 /.8/.2	1/ 0/ 0	.2/.6/.2
20p	0/.5/.5	.2/.6/.2	.2/.5/.3	.5/ 0/.5	.2/.6/.2	.3/.5/.2	.5/.3/.2	.3/.4/.3
21p	0/.3/.5/.2							
		.5/.2/.2/.1						
			.2/.2/.5/.1					
				.2/.2/.4/.2				
					.1/.2/.5/.2			
						.3/.4/.3/ 0		
							.5/.3/.2/ 0	
								.2/.3/.4/.1

There were a number of "unique" variants scored for the chromosome heteromorphic marker regions - they were found for different chromosome markers and in different collection areas. As they were just scored once, they are not included in the frequency analysis, but do increase the range of variation of potential use in further site studies.

TABLE 5: Distribution of homozygotes vs. heterozygotes for every chromosome variable region, across collection sites. Scores are indicated as the number of animals homozygous for a variant in that marker region: the number of animals heterozygous for variants in that marker region (Ho:He). The number of animals karyotyped for each collection site is indicated by "N". Not all variable regions could be scored in all karyotypes due to occasional resolution problems.

	COLLECTION SITES							
Chromosome Marker Region	IR Ho:He N=5	FK.MB Ho:He N=3	CH Ho:He N=5	TS Ho:He N=3	FPH Ho:He N=5	MS Ho:He N=3	PAC Ho:He N=3	TOTAL Ho:He N=27
1p	3:0	2:0	3:1	1:1	2:3	1:2	1:2	13:9
1q	2:1	1:1	1:1	2:1	4:1	1:2	0:3	11:10
2p	2:1	0:4	2:3	1:1	2:3	3:0	0:3	10:15
2q	0:3	2:1	4:1	2:1	2:3	0:3	2:1	12:15
3p	5:0	3:0	3:1	2:0	3:0	2:0	2:0	20:1
3q	5:0	2:1	5:0	3:0	3:1	3:0	1:1	22:3
4p	3:1	1:2	3:1	3:0	3:2	2:1	2:1	17:8
4q	3:0	0:3	4:1	3:0	3:1	1:0	2:1	16:6
5p	2:1	1:1	2:3	2:1	3:2	1:0	1:0	12:8
17q	2:3	0:2	2:3	2:1	3:1	1:1	2:1	12:12
18p	3:2	3:0	3:2	2:1	3:2	0:2	2:1	16:10
19p	1:2	2:1	0:5	2:1	4:1	2:1	2:1	13:12
20p	0:5	0:3	1:4	0:2	2:3	0:3	0:3	3:23
21p	1:4	0:3	1:4	0:3	0:5	0:2	0:3	2:24
TOTAL PER SITE	32:22	17:22	35:30	25:13	37:28	17:17	17:21	179:156

Fig. 1. The distribution of capture sites.

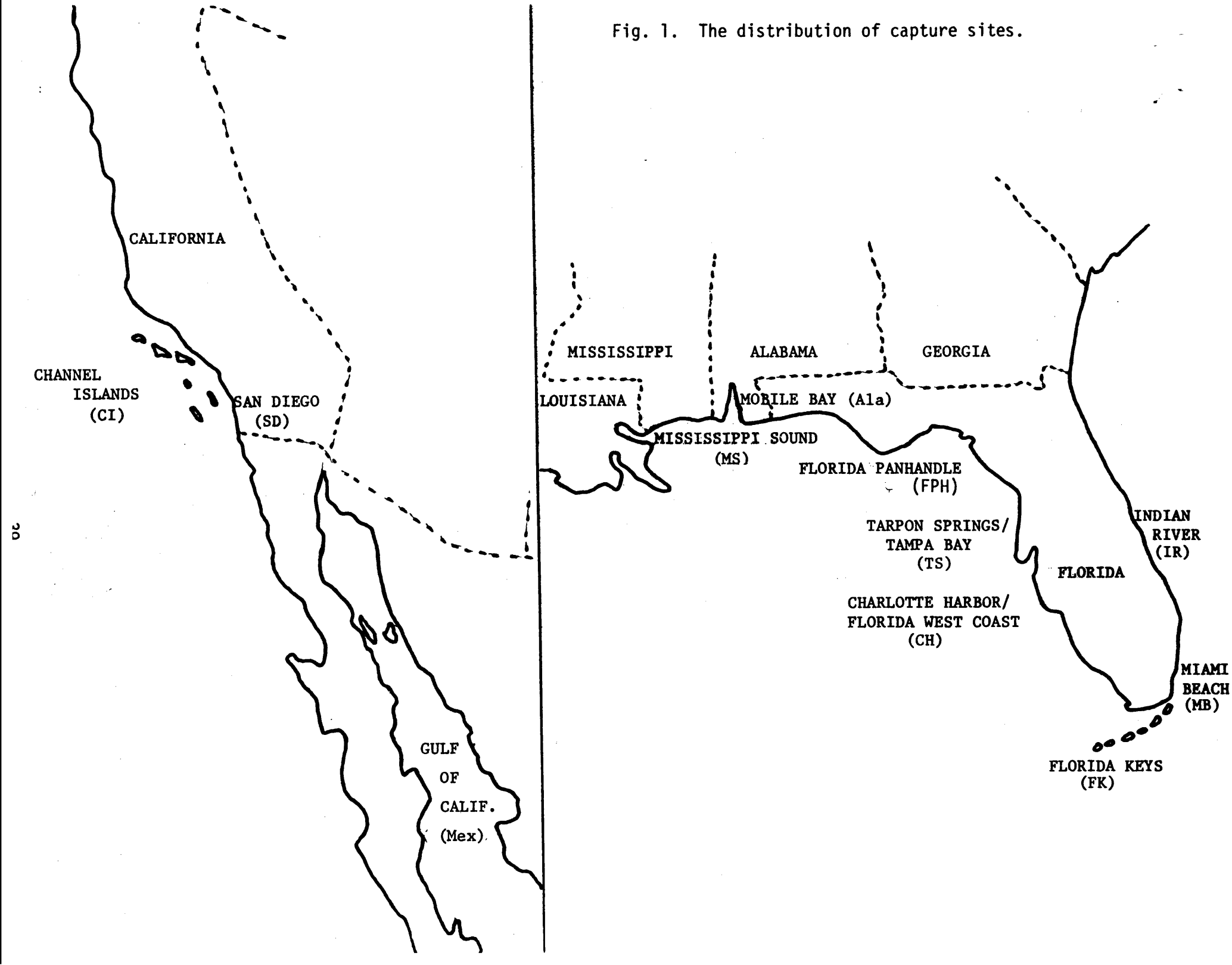
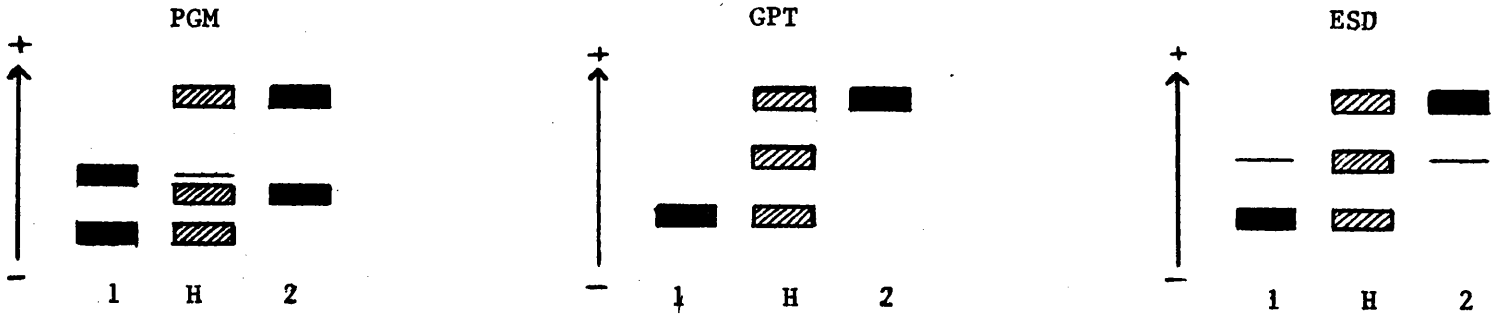
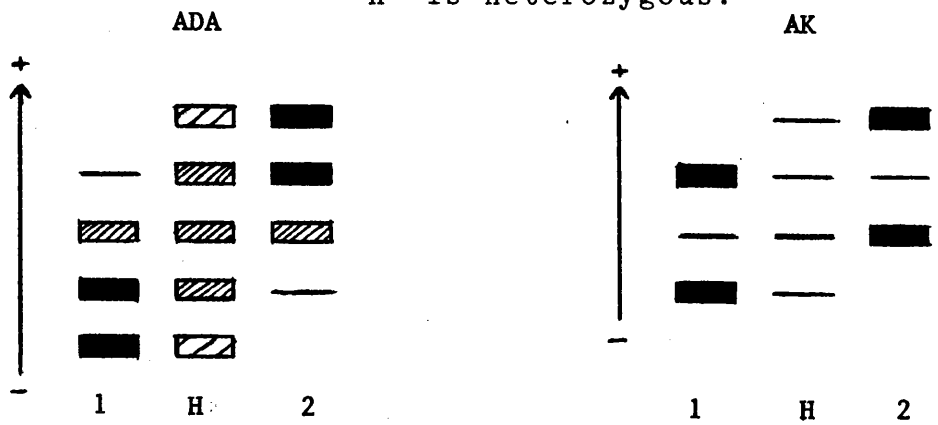


Fig. 2: Scoring systems for RBC enzymes, hemoglobin, plasma proteins and haptoglobin/ transferrin type.

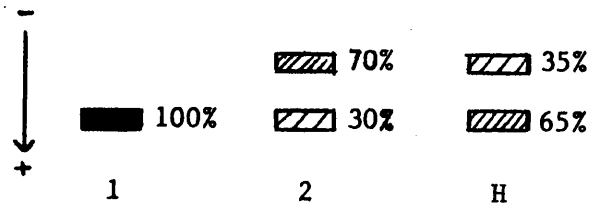
RBC ENZYMES



"1" is homozygous for the slow variant; "2" homozygous for the fast variant; "H" is heterozygous.



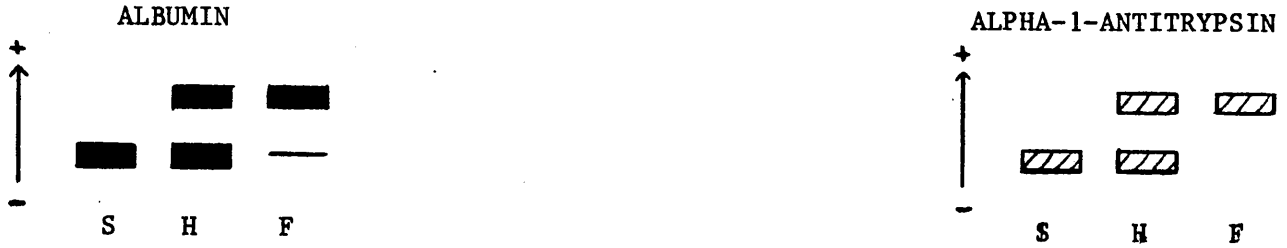
HEMOGLOBIN
Hb



"1" is the single Hb of the Atlantic Tursiops; "2" is the double Hb pattern of the Pacific Tursiops; and "H" is the pattern seen in crosses between these two types.

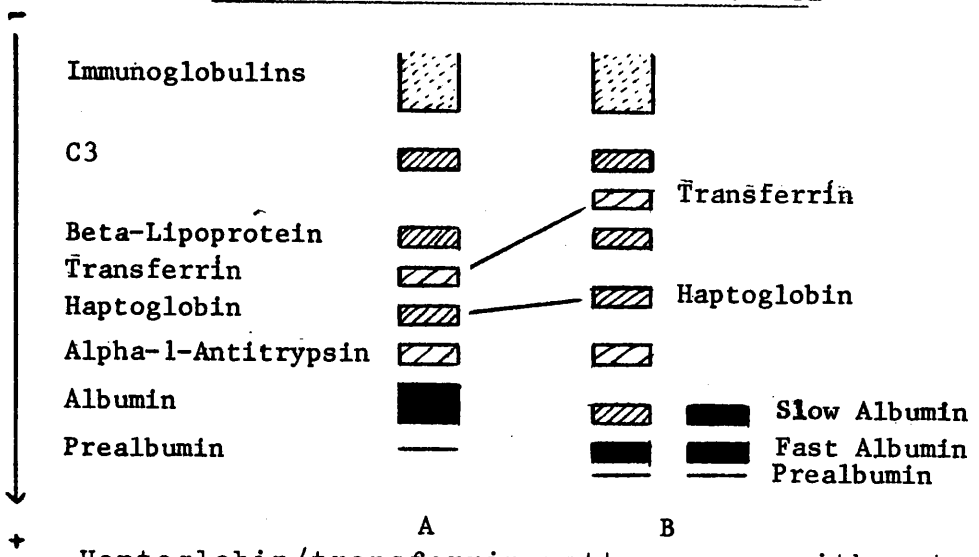
Fig. 2 (cont.)

PLASMA PROTEIN SCORING



"S" is homozygous for a slow variant; "F" is homozygous for a fast variant; and "H" is heterozygous.

HIGH RESOLUTION PATTERN ON AGAROSE



Haptoglobin/transferrin patterns are either type "A" or "B" or a combination of both, A/B.

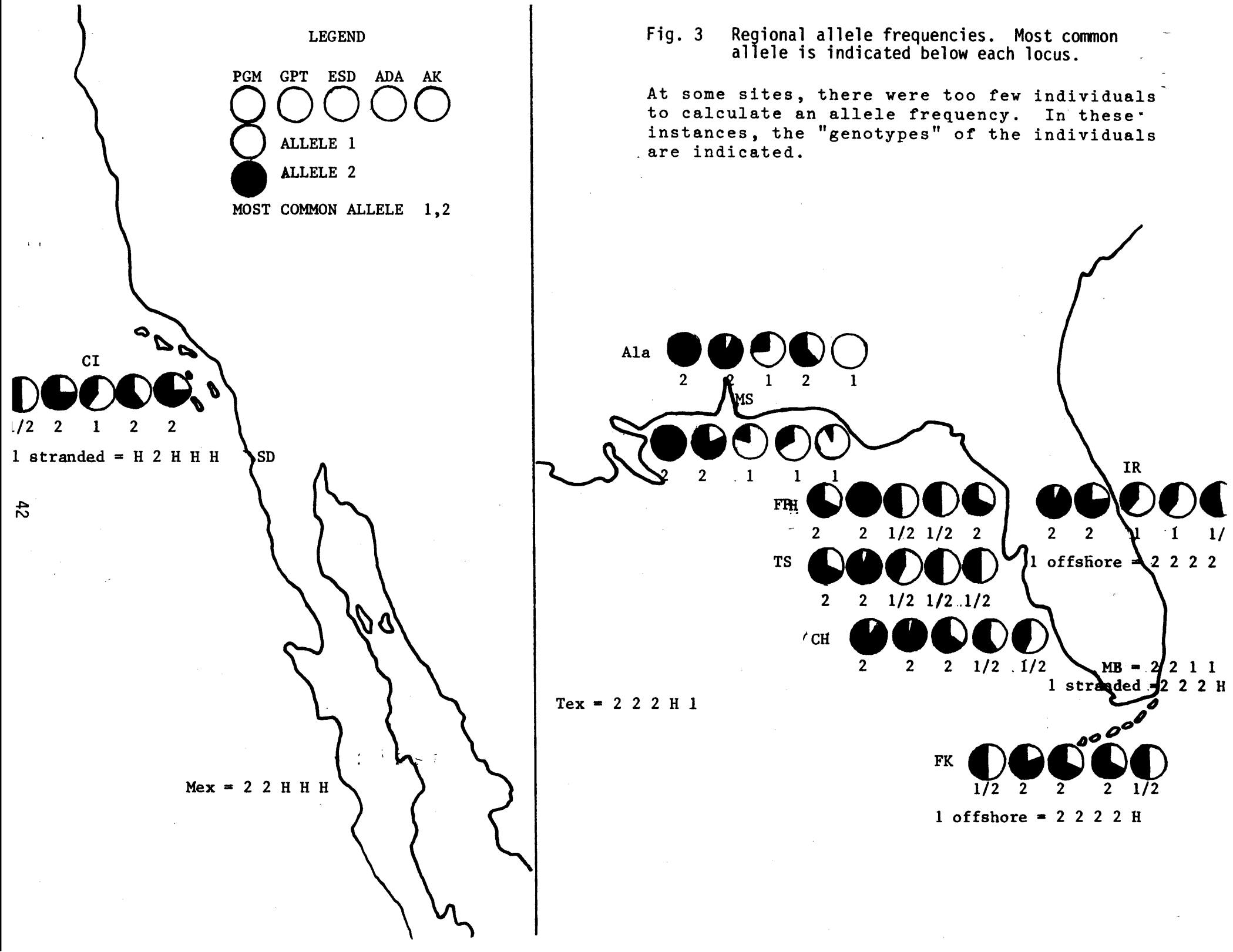
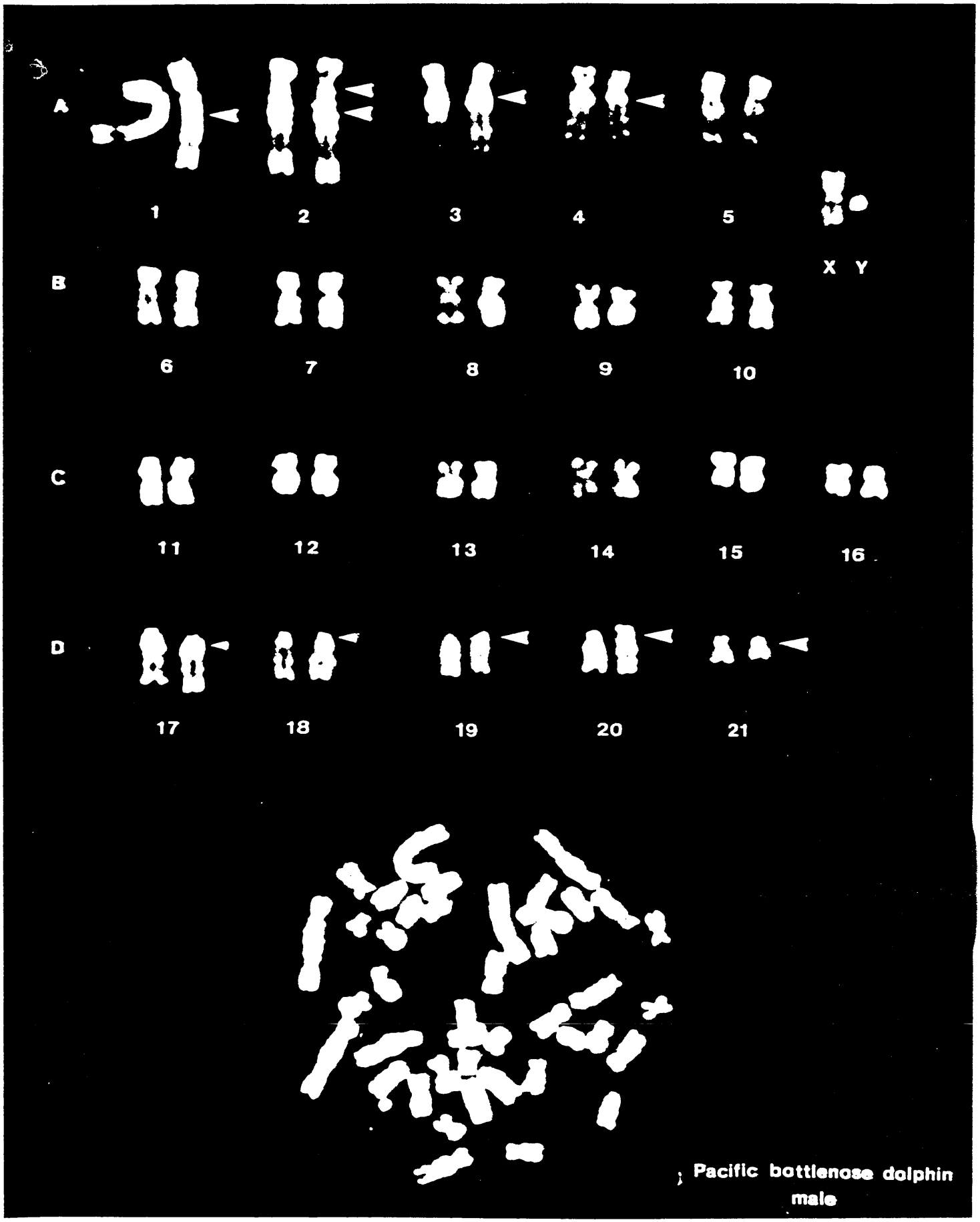


Fig. 3 Regional allele frequencies. Most common allele is indicated below each locus.

At some sites, there were too few individuals to calculate an allele frequency. In these instances, the "genotypes" of the individuals are indicated.

Fig. 4. Karyotype of a male bottlenose dolphin



Pacific bottlenose dolphin
male

Fig. 5: R-band variable regions in Tursiops.

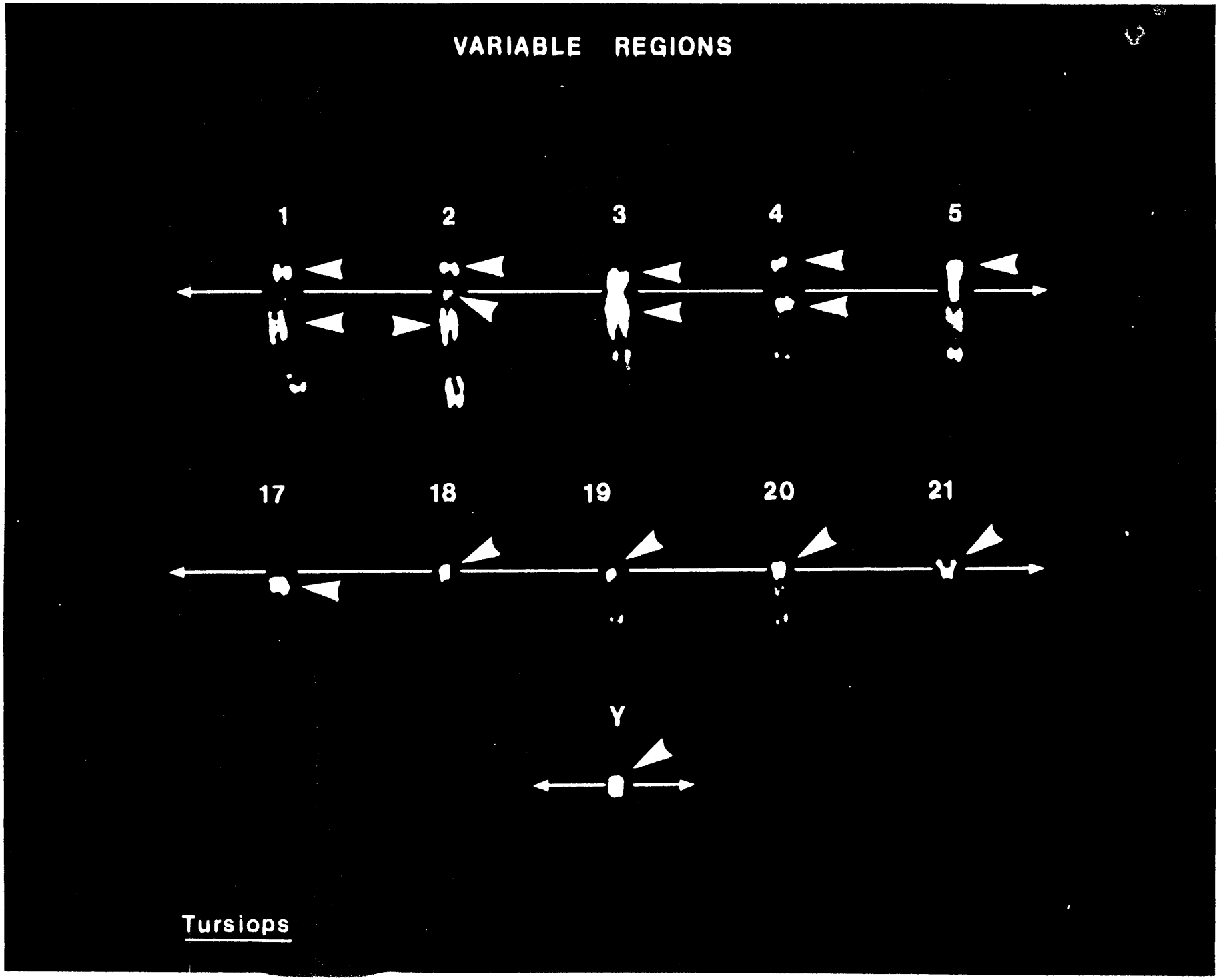
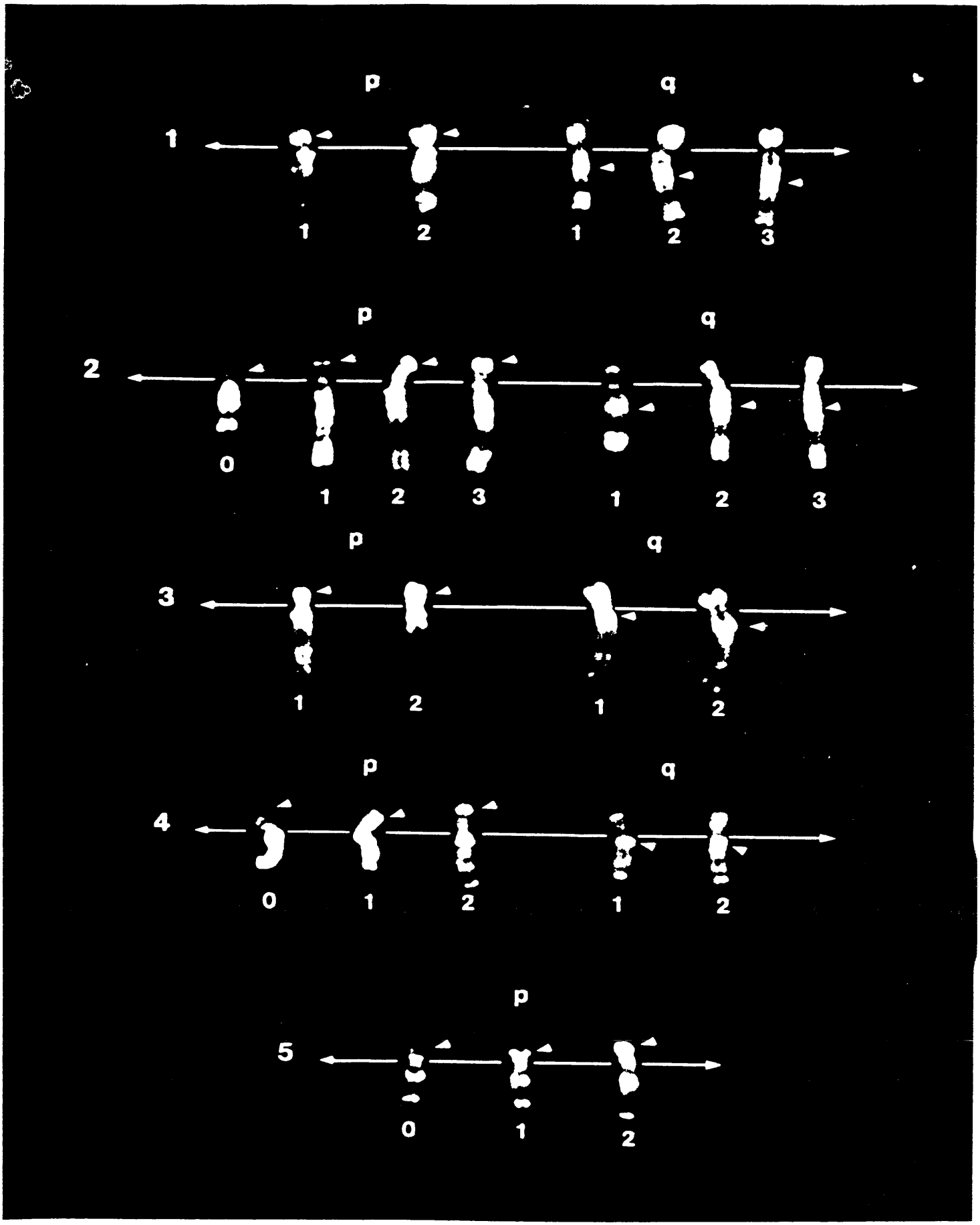

VARIABLE REGIONS
1
2
3
4
5
17
18
19
20
21
Y
Tursiops

Fig. 6: Scoring of the R-band variable regions in Tursiops. The range of markers in each heteromorphic region are scored from 1 or 0 being the smallest marker seen for that heteromorphic region in the Tursiops compared, to the largest identified. Some heteromorphic regions had as many as 7 variants detectable.


p
q
1
1
2
1
2
3
p
q
2
0
1
2
3
1
2
3
p
q
3
1
2
1
2
p
q
4
0
1
2
1
2
p
5
0
1
2

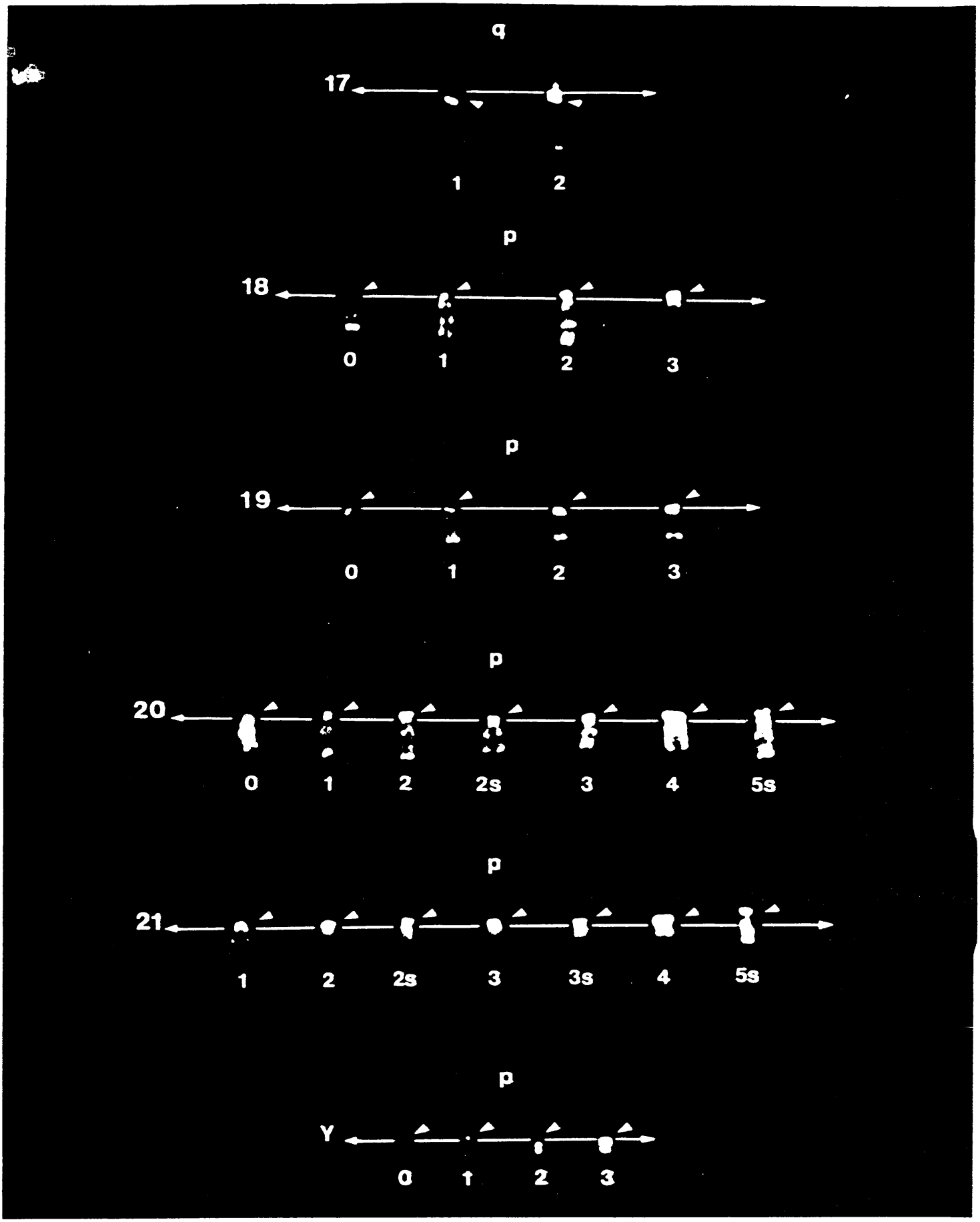
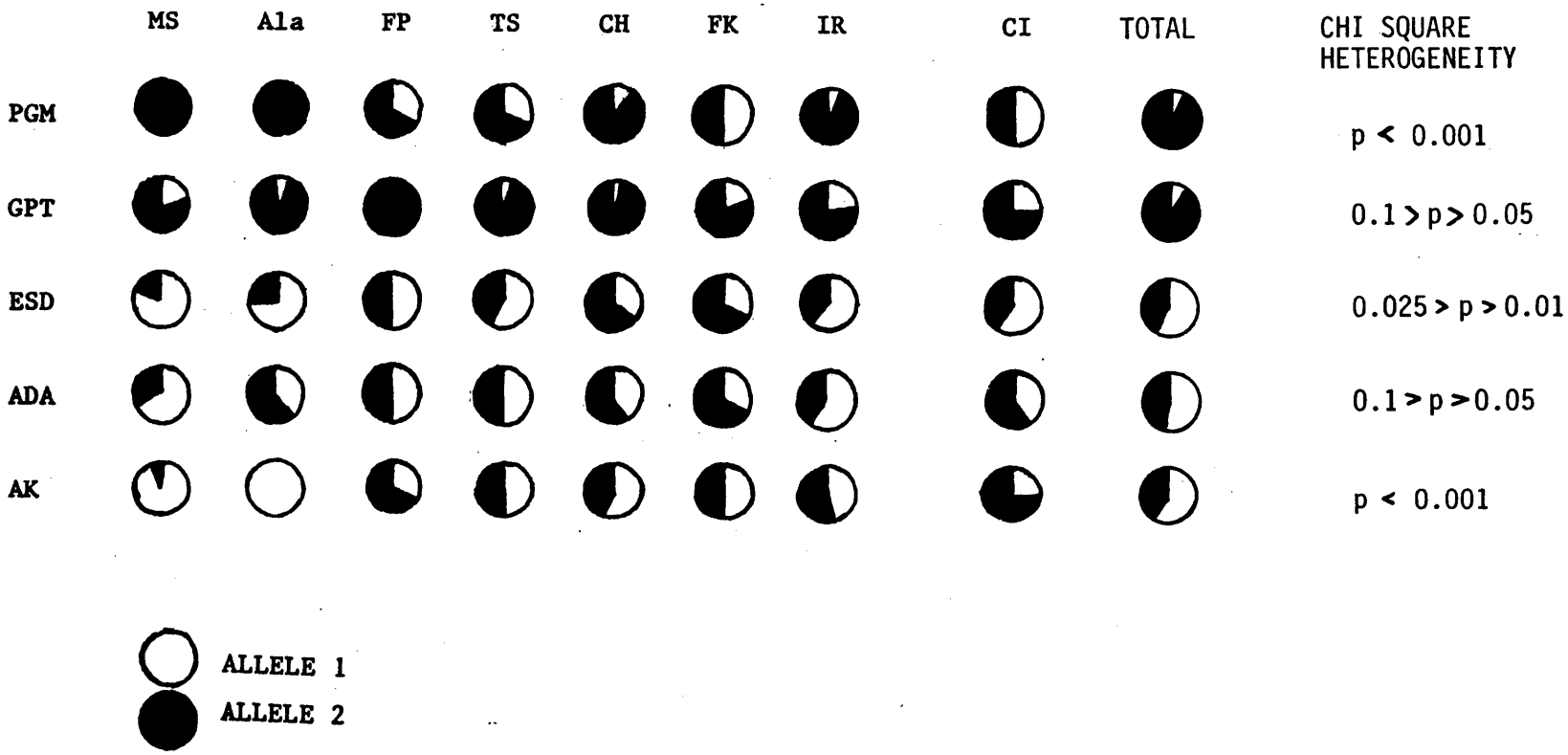

q
17
1
2
p
18
0
1
2
3
p
19
0
1
2
3
p
20
0
1
2
2s
3
4
5s
p
21
1
2
2s
3
3s
4
5s
p
Y
0
1
2
3

Fig. 7 REGIONAL ALLELE FREQUENCIES



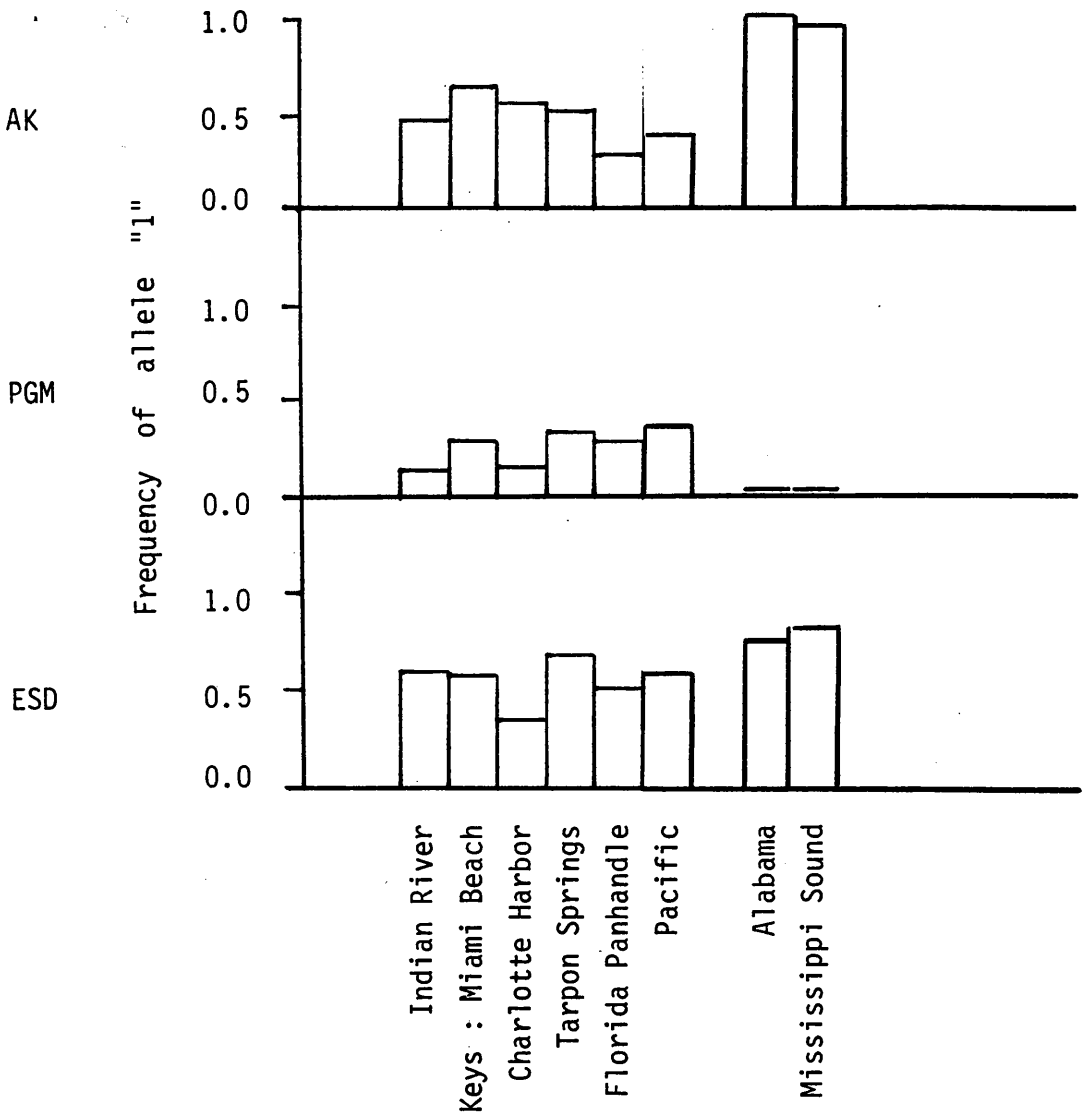


Fig. 8 Frequency of allele "1" across collection sites for the three enzymes which showed significant between sample heterogeneity (AK, PGM, ESD). Pooling of the Alabama and Mississippi Sound samplings separately from the other samplings (for all three loci) eliminated heterogeneity. In addition, Florida Panhandle pooled separately for AK and Charlotte Harbor for ESD.

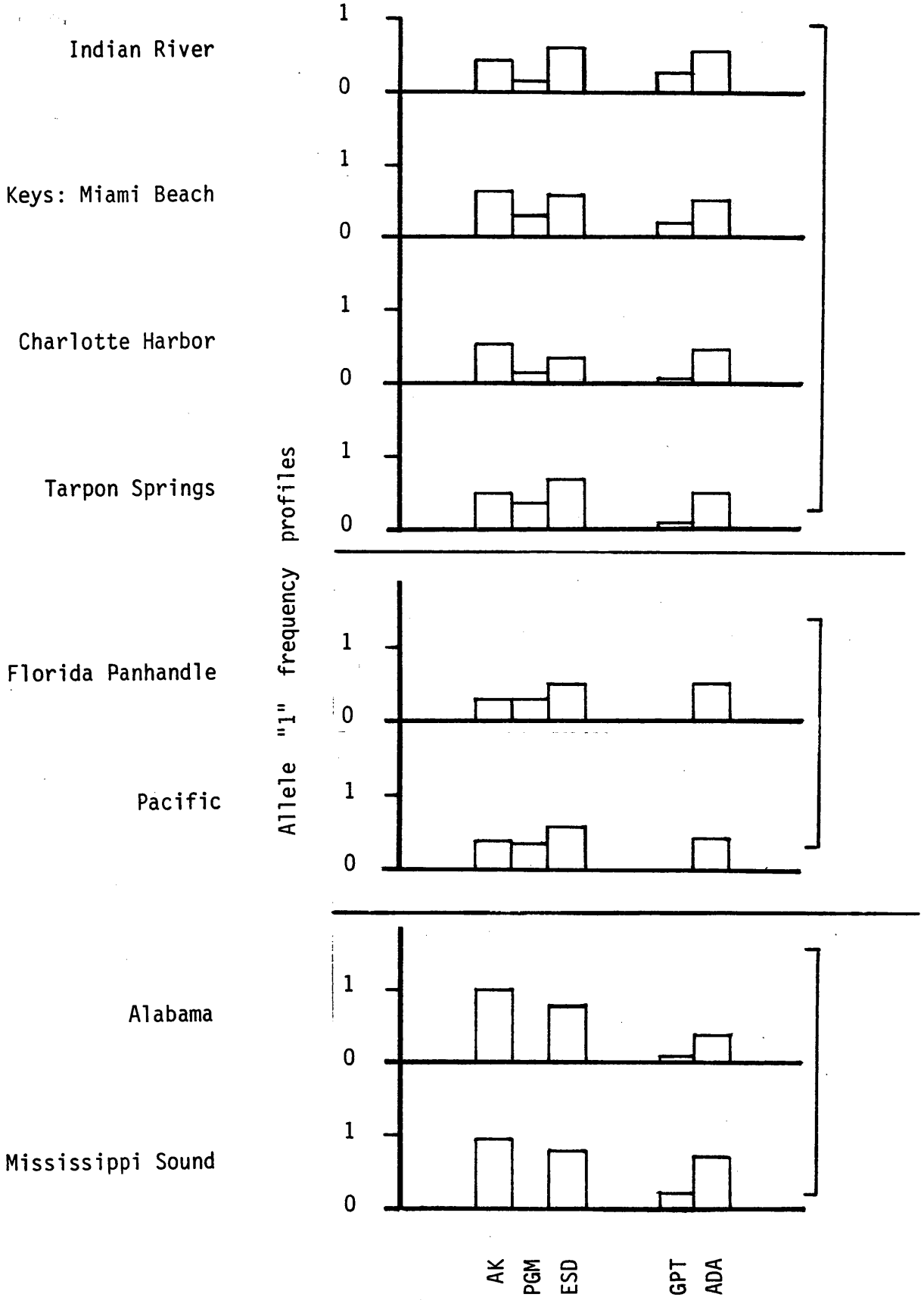
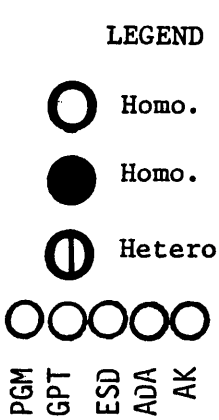
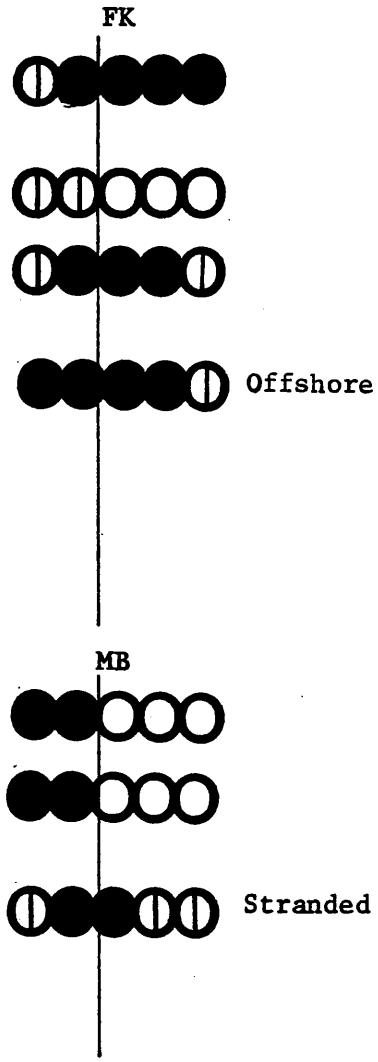


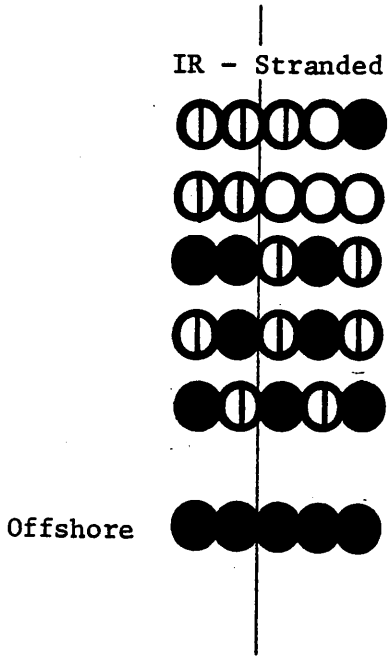
Fig. 9 Grouping of allele "1" frequency profiles for all five protein loci by collection site.

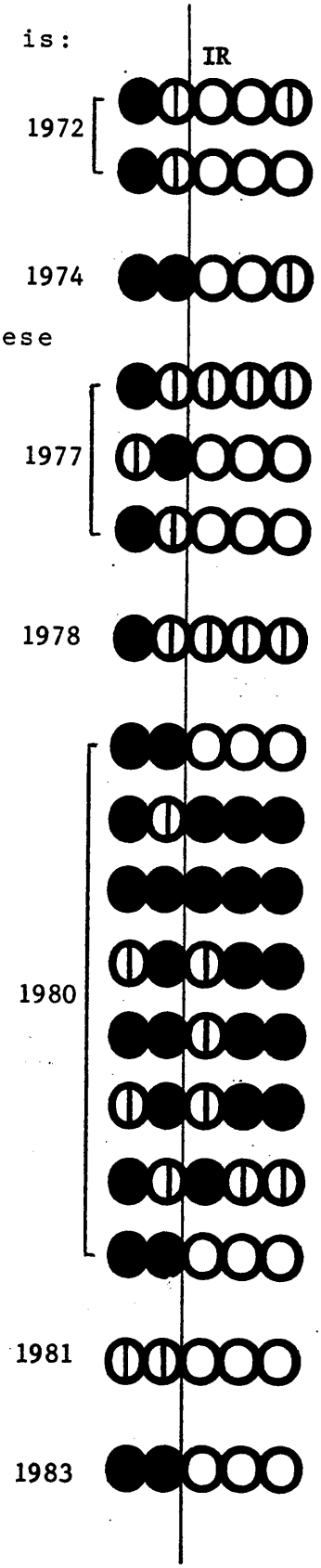
Fig. 10
INDIVIDUAL GENOTYPES



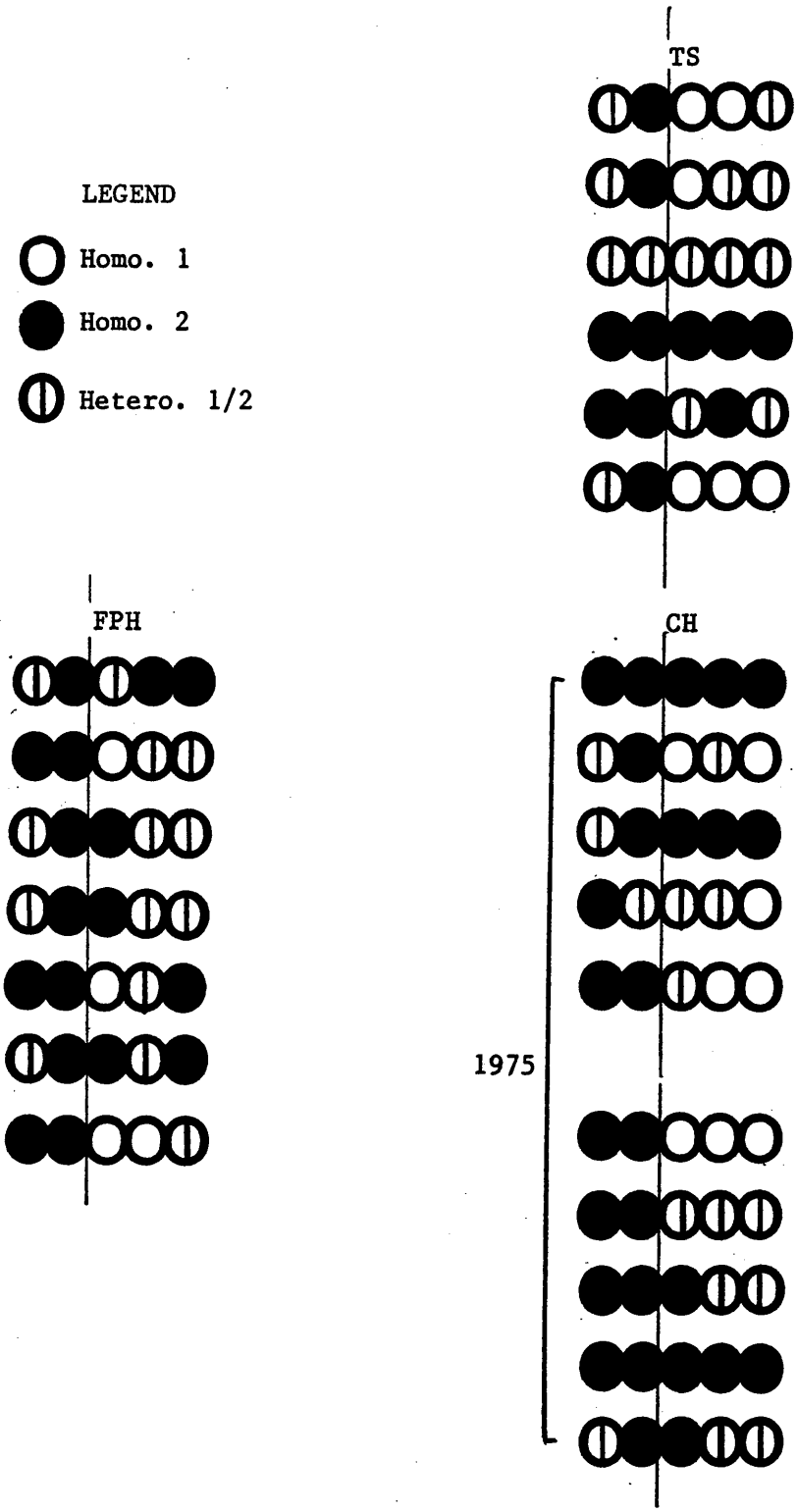
The sequence of protein loci is:
PGM-GPT-ESD-ADA-AK
Each is scored as indicated in the legend. PGM and GPT do not show a striking distribution pattern, but the three loci to the right of the line do. Note the distribution of "1 1 1" genotypes, "2 2 2" genotypes and "H H H" genotypes for these three protein loci.







INDIVIDUAL GENOTYPES



INDIVIDUAL GENOTYPES